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THE FOODS AND FEEDING HABITS OF THE SAVANNAH SPARROW IN WINTER

By Thomas L. Quay

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Introduction and Methods.—The savannah sparrow (*Passerculus sandwichensis* Gmelin) is a common winter resident in various herbaceous communities in the southeastern states. Winter populations of this sparrow in the lower Piedmont of North Carolina vary from some fraction of one per acre in certain tall-weed and broomsedge (*Andropogon*) fields to fifteen per acre in well-developed crabgrass (*Digitaria*) fields (Quay, 1947, 1957). The population density in other areas may be even higher (Norris and Hight, 1957). The habitat requirements of the savannah sparrow on winter range are: (1) bare ground, on which to move and forage, (2) an abundance of small seeds, easily available on the ground surface without scratching, and (3) an overhead cover of herbaceous vegetation (Quay, 1957). In the vicinity of Raleigh, N. C., at the east-central edge of the lower Piedmont, these essential environmental factors are best supplied in those fields in which *Digitaria* is common to dominant (*ibid.*). It is to be expected that similar favorable habitat in other localities will be found in communities of somewhat different plant composition (Odum, 1940: 197).

Previous studies on the food habits of savannah sparrows have been few and scattered, with none pertaining to the Southeast (Martin, Zim, and Nelson, 1951). The present study was conducted as part of a broader investigation on habitat selection by wintering old field sparrows. One hundred savannah sparrows were collected in each winter (December, January, and February) of 1946–47 and 1947–48. The specimens were shot on established plots, apportioned among the different stages of the old field plant succession, in agricultural land bordering Raleigh, N. C., on the west. The 200 stomachs were analyzed by the aggregate percentage method (Martin, Gensch, and Brown, 1946). Stomach, in food-habits literature, means "the material from either crop or gizzard, or the combined content of the two" (Stoddard, 1931: 114) and is so used in this paper. Reference is also made to either crop or gizzard alone.

Grateful acknowledgment is made to Dr. Alexander C. Martin for material aid in seed identification and for personal instruction in the methodology of food-habits analysis. Several fruitful days in August of 1947 were spent under Dr. Martin's guidance in the Economic Investigations Laboratory of the United States Fish and Wildlife Service, Patuxent Research Refuge, Laurel, Maryland.

Plant Foods and Feeding Habits. *General.*—The over-all averages of the kinds and amounts of foods eaten are listed in Table 1, grouping the variations due to plot, date, hour, and weather. This is probably the most valid type of analysis, since collections were spread throughout the day and winter and were roughly in direct proportion to the populations in the respective plots. In consideration of the differences between the two winters in weather, population density of sparrows, and vegetative composition of the plots, the two sets of totals and averages were remarkably similar. The low number of 3.6 items per stomach revealed a narrow selection. The high volume of seeds eaten, approximately 97 per cent in both years, clearly demonstrates the savannah sparrow to be almost exclusively herbivorous in the winter season.

Seeds Taken.—In this paper the word "seed" is used in both a general sense (including caryopsis and achene) and the specific botanical sense. The full list of all species of seeds found in the 200 savannah sparrow stomachs is given below, in alphabetical order. The scientific terminology follows that of Gray's Manual of Botany, eighth edition (Fernald, 1950). The figures represent

TABLE 1

Average volume and frequency of food items in 200 savannah sparrow stomachs, winters of 1946–47 and 1947–48

Food Item	1946-47 (100 stomachs)		1947-48 (100 stomachs)	
	Volume	Frequency	Volume	Frequency
Seeds				
Digitaria	71.90	100	68.68	98
Sorghum	11.66	32	3.74	11
Eleusine	3.35	19	2.91	26
Ambrosia	3.00	50	8.77	61
Aristida	1.75	2	0.35	2
Andropogon	1.73	10	2.50	6
Aster	0.78	6	1.00	8
Amaranthus	0.72	12	2.38	12
Diodia	0.55	1	—	—
Mollugo	0.53	19	—	—
Cyperus	0.43	11	2.46	17
Setaria	0.35	5	1.47	7
Eragrostis	0.27	5	—	—
Oenothera	0.20	2	0.28	9
Stellaria	0.16	3	—	—
Croton	0.15	1	0.10	5
Cynodon	0.05	1	—	—
Muhlenbergia	0.05	1	—	—
Panicum	0.04	2	0.06	3
Paspalum	0.04	1	0.42	3
Polygonum	0.04	1	0.34	3
Euphorbia	Trace	1	0.06	4
Oxalis	—	—	0.30	5
Glycine	—	—	0.25	1
Subtotal	97.75	285	96.07	281
Arthropoda				
Coleoptera	1.31	29	2.72	35
Insect Eggs	0.56	32	0.31	9
Unidentified	0.13	6	0.06	6
Collembola	0.13	3	Trace	2
Homoptera	0.10	6	0.35	12
Araneida	0.02	1	0.16	4
Lepidoptera	—	—	0.27	7
Diptera	—	—	0.05	1
Hemiptera	—	—	0.01	1
Subtotal	2.25	77	3.93	77
Total	100.00	362	100.00	358
Average Frequency		3.62		3.58

three-dimensional measurements of seed-size in millimeters. In every case, the measurements were of "naked" seeds (glumes, lemma, and palea removed from grass seeds, the pericarp removed from dicotyledonous seeds), the condition in which the seeds were usually found in the savannah sparrow crop. All the seeds were of a uniformly small size, with the upper limit of the greatest dimension being at about 3.0 mm. (except *Glycine*; taken once, in pieces). The list gives twenty-four genera, of which fifteen are represented by a single species, four have only two species each (the two in each case being alike in size and growing side by side), two have a single main species and two or three others indistinguishable in stomachs, and three have from two to four species of similar appearance and uncertain identity in stomachs. Thus, the total number of species of seeds taken was about thirty-six. Because of this arrangement, the genus was selected as the simplest and most valid category to use in the table and descriptions.

1. *Amaranthus* spp. Amaranth. 1.0 × 0.8 × 0.4.
2. *Ambrosia artemisiifolia* L. Common Ragweed. 2.0 × 1.3 × 1.0.
3. *Andropogon* spp., especially *A. virginicus* L. Broomsedge. 2.0 × 0.4 × 0.3.
4. *Aristida oligantha* Michx. Prairie Three-awn. 4.0 × 0.4 × 0.3. Always broken into two pieces before swallowed.
5. *Aster* spp., especially *A. pilosus* Willd. Aster. 1.0 × 0.4 × 0.3.
6. *Croton glandulosus* L. Croton. 3.0 × 2.5 × 1.5. No whole seeds were ever found.
7. *Cynodon dactylon* (L.) Pers. Bermuda Grass. 1.9 × 0.7 × 0.5.
8. *Cyperus strigosus* L. Galingale. 1.5 × 0.5 × 0.5.
9. *Cyperus compressus* L. Galingale. 1.2 × 1.0 × 1.0.
10. *Digitaria sanguinalis* (L.) Scop. Crabgrass. 2.0 × 0.8 × 0.4.
11. *Digitaria ischaemum* (Schreb.) Muhl. Smooth Crabgrass. 1.5 × 0.7 × 0.4.
12. *Diodia teres* Walt. Buttonweed. 2.5 × 1.8 × 0.9.
13. *Eleusine indica* (L.) Gaertn. Goosegrass. 1.2 × 0.7 × 0.6.
14. *Eragrostis hirsuta* (Michx.) Nees. Bigtop Lovegrass. 0.8 × 0.5 × 0.5.
15. *Eragrostis elliottii* S. Wats. Elliott Lovegrass. 0.7 × 0.3 × 0.3.
16. *Euphorbia maculata* L. Spotted Euphorbia, Eyebane. 1.0 × 0.7 × 0.7.
17. *Glycine max* (L.) Merr. Soybean. 9.0 × 6.0 × 3.5. Whole seed not swallowed, but torn into many small parts; taken only once.
18. *Mollugo verticillata* L. Carpetweed. 0.6 × 0.5 × 0.3.
19. *Muhlenbergia schreberi* J. F. Gmel. Nimblewill. 1.3 × 0.4 × 0.4.
20. *Oenothera laciniata* Hill. Cutleaf Evening Primrose. 1.1 × 0.8 × 0.7.

21. *Oxalis stricta* L. Common Yellow Oxalis. 1.2 × 0.7 × 0.2.
22. *Panicum* spp. Panicum. 2.4 × 1.2 × 0.7.
23. *Paspalum* spp. Paspalum. 2.0 × 1.5 × 0.7.
24. *Polygonum pensylvanicum* L. Pennsylvania Smartweed, Pinkweed. 3.0 × 2.5 × 1.0.
25. *Polygonum persicaria* L. Spotted Ladysthumb. 2.0 × 1.4 × 0.8.
26. *Setaria glauca* (L.) Beauv. Foxtail. 2.0 × 1.4 × 0.8.
27. *Sorghum halepense* (L.) Pers. Johnson Grass. 2.4 × 1.6 × 1.0.
28. *Stellaria media* (L.) Cyrillo. Chickweed. 1.5 × 1.3 × 0.7.

Relative Importance.—*Digitaria* seeds formed 70 per cent of all food eaten. *Digitaria sanguinalis* was the common species, being at least ten times more abundant in the field than *Digitaria ischaemum*. The two plants had the same growth form and occurred together in the same field. The seeds were only slightly different in size, and were taken by the savannah sparrows in about the same proportion to each other as they existed in the plots. *Digitaria* seeds occurred in all but two stomachs, and in many stomachs composed 90–100 per cent of the contents.

The second most important seed was *Ambrosia artemisiifolia*, with frequencies of 50 and 61 but average percentage volumes of only 3.00 and 8.77. The increase of 5.77 per cent from the first winter to the second was not high in view of the increase of the plant in several plots from uncommon to codominant. Thus, *Ambrosia* was taken often but usually in low amount.

The volumes and frequencies of *Sorghum halepense* seeds were regulated almost entirely by the number of specimens collected in the several acres of this grass in Plot 9. In this small patch, in which *Digitaria* was codominant in 1946–47 and common in 1947–48, the collections numbered 23 and 9; for these the frequencies both winters were 100 and the average volumes about half *Sorghum* and half *Digitaria*.

Eleusine indica occurred commonly in all *Digitaria* fields, especially the second year. Its seeds ranked along with those of *Ambrosia* and *Sorghum* as a food of secondary importance. As with *Sorghum*, both volume and frequency were relatively low.

Genera of tertiary importance (average volume more than one per cent in at least one winter but always less than three per cent) were: *Andropogon, Cyperus, Amaranthus, Setaria, Aristida.*

Genera of quaternary importance (average volume less than one per cent both winters) were: *Aster, Diodia, Mollugo, Eragrostis, Oenothera, Stellaria, Croton, Cynodon, Muhlenbergia, Panicum, Paspalum, Polygonum, Euphorbia, Ozalis, Glycine.*

Seed Availability.—Since the savannah sparrow was normally limited to feeding on the ground, the number of seeds present and available there was important. A planned statistical analysis of such availability through the winter would have been valuable, but was not done. However, some notes were made by observation and estimate on the percentage loss (shatter) of seeds from the plants and the number of seeds easily visible on the ground.

Most of the annual dicotyledonous species had lost all of their seeds by the beginning of winter. Many of these were small and soon became covered with dirt and litter. *Setaria* shattered early in the fall. Most of the seeds of tertiary and quaternary importance were 60–95 per cent shattered by the beginning of winter and 95–100 per cent so by mid-February. Many of these plants were either low in numbers (as *Polygonum, Paspalum, Panicum*, etc.) or had small and light seeds which scattered diffusely (*Aster* and other kinds of tall weeds). *Cyperus strigosus* was 40 per cent shattered by January 1, but still retained 10–15 per cent of its seeds by March 1.

The seeds of secondary importance shattered later in the season and over a longer time than the ones of lesser use. *Ambrosia* was 50–60 per cent shattered by January 1 and 95–100 per cent so by March 1, but retained a good supply of seeds most of the winter. *Ambrosia* seeds on the plant were a primary source of food to all old field sparrows when snow was on the ground. *Sorghum halepense* likewise held its seeds well for most of the winter. It was 60 per cent shattered by January 1, but had some seeds until the end of February. *Eleusine indica* was slow to shatter, there being 50 per cent of the seeds still on the plants by March 1.

Digitaria was a late-summer and fall plant, the seeds maturing late. Shattering was possibly 50 per cent complete by January 1 and 90–95 per cent complete by March 1. All winter long there was a steady dropping of fresh seeds onto the ground surface. Throughout the winter, in areas where *Digitaria* was common to dominant, the ground below had from 100 to 1000 *Digitaria* seeds per square foot easily visible on the surface. The supply did not seem ever to diminish appreciably. It was not likely that the supply of *Digitaria* seeds ever became limiting in fields where *Digitaria* was well-developed and dominant. On

the other hand, it was most likely that the low to negligible supply of *Digitaria* seeds in other fields was an important limiting factor on savannah sparrow populations there.

Andropogon, like *Eleusine*, still had at least 50 per cent of its seeds on the plant at winter's end. The low number of seeds on the ground in *Andropogon* fields probably was a critical limiting factor to savannah sparrows. Field sparrows (*Spizella pusilla*), however, were much at home in *Andropogon* and regularly picked seeds directly from the plant. *Amaranthus* produced a great abundance of seed, much of which stayed on the plant all winter but very little of which was ever taken by savannah sparrows.

Feeding Behavior.—Savannah sparrows proved to be very localized feeders. Both the observation of birds feeding and the laboratory analysis of the stomachs revealed that full meals were usually made primarily on some one to four kinds of seeds. It became possible to tell, without looking at the collection data, in which plot and even which part of the plot a bird had been feeding. Practically all the plots, especially during the second winter, were characterized by a patchy arrangement of some of the plants. The sparrows moved very little from one plot to another, or even from one part of a plot to another part, during the course of any one feeding period. Fully 90 per cent of all stomachs contained only those kinds of seeds known to be present in the local area (1–5 acres) where the bird had been shot. The savannah sparrows ate what was available to them wherever they were. In Plot 1, an *Andropogon* field with *Aristida* on eroded spots, the few savannah sparrows present ate primarily *Andropogon* and *Aristida* seeds. In Plot 3, where many kinds of herbaceous old field plants were available, the few savannah sparrows present made a wider than usual selection of seeds. In the *Digitaria-Medicago* (alfalfa) plots, which had the fewest kinds of plants present as well as the narrowest selection of seeds by the birds, the populations were mediumly large but *Digitaria* seeds formed about 90 per cent of the total food, the rest being mostly *Eleusine* seeds and insects. Plot 9 had the best development of *Digitaria* both years and also the highest average number of savannah sparrows per acre. The average volumes of *Digitaria* seeds eaten by the savannah sparrows collected in Plot 9 were 73.65 per cent the first winter and 65.08 per cent the second, as compared with the over-all averages of 71.90 per cent and 68.68 per cent. It is evident that by selection of specimens many different results could be obtained. For instance, if all collections were made only in *Andropogon* fields, the food-habits results would likely indicate an average volume of 90 per cent *Andropogon* seeds. Thus, one key to habitat preference or selection by savannah sparrows (or other species) is the study of food habits in the most heavily populated habitats.

The savannah sparrow typically fed on the ground, picking up seeds from the ground like a chicken. The only times that they were seen to take seeds directly from plants were when snow and sleet covered the bare ground. The other kinds of sparrows commonly and regularly picked seeds directly from upright and prostrate plants. Savannah sparrows did not pick seeds even from prostrate *Digitaria* as long as there was bare ground available under or by some overarching *Digitaria*. Following rains, savannah sparrow beaks were always muddy and more than the usual amount of soil was found adhering to the seeds in the crops.

The seeds of grasses fall off the plant usually as single-seeded spikelets, as in the tribe Paniceae (*Digitaria, Setaria, Panicum, Paspalum*), or as individual florets (*Eleusine*). The savannah sparrows hulled each grass seed before swallowing it; that is, the glumes and/or lemma and palea were first removed. For all those seeds having indurated lemmas and paleas (Paniceae, *Sorghum*), this should have been quite a difficult feat but nevertheless was accomplished with seeming ease and rapidity. An occasional seed was swallowed with either palea or both palea and lemma still attached. Similarly, the hardened ovarian or carpellary walls (pericarp) were usually cracked and removed from the dicotyledonous seeds before they were swallowed (especially *Ambrosia, Croton, Diodia*). This more delicate method of feeding was in contrast to that of larger birds such as meadowlarks and mourning doves which swallowed entire ovaries, florets, and single-seeded spikelets (Cummings and Quay, 1953).

Table 2 gives the average volume of the stomachs collected during each hour of daylight. Feeding apparently began a few minutes before sunrise and lasted until about a half-hour after sunset. There was some feeding all day long, but the crop was used for storage only once a day, toward sunset. There were some entirely fresh or only partially digested seeds in the gizzard at all times of the day. The data from the stomachs substantiated the field observations that feeding during the hours of daylight was a continuous,

though at times unhurried, process. In the morning, feeding was most active during the first two hours of light but no crop ever had anything in it. In the afternoon, some crops began to fill about two hours before sunset. The main period of markedly accelerated feeding and crop-filling was from one hour before sunset until dark.

Separate measurement of crop and gizzard volumes of stomachs collected within the last two hours before sunset indicated that the crop did not ever contain anything until the gizzard was either full (0.20cc.) or stuffed (0.30cc.). A crop was full at 0.30cc. and stuffed at 0.40cc. Total stomach capacity was 0.70cc. In the more favorable *Digitaria* fields (100–1000 seeds on the ground surface per square foot, at least in spots), savannah sparrows apparently had no difficulty in stuffing the gizzard and crop by the end of the evening twilight; at this more critical time and place the percentage of *Digitaria* seeds taken was higher than average. The largest number of *Digitaria* seeds found in any one stomach was 955, there being 813 just in the crop (and esophagus). Instances of over 500 seeds per stomach were common.

All the measurements of stomach volumes included the grit. Grit was renewed each day, typically. It ranged in amount up to about 0.10cc., and was considered too small in proportion to alter significantly the volumetric data.

Within the spread of normal weather conditions, there were no noticeable shifts in food habits during the course of either winter. *Digitaria* seeds began and ended as the outstandingly important item. The greatest deviations occurred during the distinctly unusual three weeks of nearly continuous sleet, snow, and ice glaze between January 24 and February 14. These conditions practically eliminated savannah sparrow habitat from the region for the period. There resulted a marked reduction in the local savannah sparrow population, apparently by a strong movement southward (Quay, 1957). The few remaining savannah sparrows, which may have been individuals freshly arrived from more northern areas, continued to approximate regular feeding habits insofar as conditions allowed, but also picked some seeds directly from the plants (*ibid.*).

ANIMAL FOOD.—The animal food taken was relatively unimportant, at least in amount if not in nutritional value. It was all composed of insects, insect eggs, and spiders (Table 1). Identifications were made only to order. Coleoptera, larvae and adults, composed more than half the total each winter. There were four or more kinds of insect eggs, most of which presumably were of Orthoptera and about the size of *Digitaria* seeds. The sources of these eggs were not discovered. The other orders were: Homoptera, Lepidoptera (larvae), Collembola, Diptera (larvae), Hemiptera, Araneida.

TABLE 2

Stomach contents from sunrise to sunset of 188 savannah sparrows, winters of 1946–47 and 1947–48

Time in Hours	Number of Stomachs	Average Volume In CC	Range of Volume In CC
After Sunrise			
0–½	14	0.09	0.04–0.20
½–1	16	0.12	0.05–0.25
1–1½	18	0.11	0.05–0.20
1½–2	11	0.09	0.05–0.15
2–3	44	0.13	0.05–0.20
3–4	11	0.14	0.05–0.30
4–5	9	0.14	0.10–0.20
Before Sunset			
5–4	5	0.19	0.15–0.20
4–3	7	0.23	0.10–0.35
3–2	7	0.20	0.10–0.30
2–1½	6	0.18	0.10–0.30
1½–1	5	0.24	0.10–0.45
1–½	12	0.38	0.10–0.70
½–0	23	0.37	0.15–0.70

Insects were taken mostly at times of higher temperatures, as would be expected. This was early in December, late in February, and on warm days in mid-winter. Even so, insect-taking was limited mainly to the middle of the day.

It is believed that insects were captured and eaten in direct proportion to their abundance and availability in the particular spots where savannah sparrows were otherwise feeding on seeds. This was the thesis of McAtee (1932: 144), who believed that selection of animal prey by birds was in proportion to abundance.

SUMMARY

Two hundred savannah sparrows were collected from various herbaceous communities in the vicinity of Raleigh, N. C., during the winters of 1946–47 and 1947–48. The stomach contents were analyzed for taxonomic and volumetric content, using the aggregate percentage method.

Total foods averaged 97 per cent seeds and 3 per cent insects and spiders. *Digitaria* seeds formed 70 per cent of all foods eaten, being easily the single most important item throughout the season (December 1–February 28) and especially toward dusk each day. Seeds of secondary importance were *Ambrosia*, *Sorghum*, and *Eleusine*. Seeds of tertiary importance were *Andropogon*, *Cyperus*, *Amaranthus*, *Setaria*, and *Aristida*. Fifteen additional genera constituted the remaining seeds eaten, each in less than one per cent volume. The seeds of greater use shattered from the plants later and more gradually than the ones of lesser use, thus being more steadily available both on the plant and on the ground. The details of the shattering progression for the different species are described. Feeding was characteristically local at any one period, on some one to four kinds of seeds. Food items were normally picked from the surface of the bare ground, under an overhead cover of foliage. Glumes, lemmas, and paleas were removed from grass seeds before swallowing, and hardened pericarps cracked off dicotyledonous seeds. Even the most indurated coverings were removed with seeming ease and rapidity from seeds up to 3.0 mm. in the greatest dimension. Seeds larger than 3.0 mm. were rarely taken and these were first broken into smaller pieces before swallowing. The de-glumed seed of *Digitaria sanguinalis*, the prime food item, measured 2.0 x 0.8 x 0.4 mm. Feeding was a continuous process, unhurried during most of the day but accelerated early in the morning and late in the afternoon. The crop was filled only once a day, at sunset.

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PROCAMBARUS PEARSEI PLUMIMANUS, A NEW CRAYFISH FROM NORTH CAROLINA (DECAPODA, ASTACIDAE)[1]

By Horton H. Hobbs, Jr. and Margaret Walton[2]

Only four crayfishes belonging to the genus *Procambarus* have been reported previously from North Carolina, *P. blandingii blandingii* (Harlan, 1830: 229), *P. blandingii acutus* (Girard, 1852: 91), *P. lecontei* (Hagen, 1870: 45), and *P. pearsei* (Creaser, 1934: 1). The relationships of the former two have never been determined adequately; however, it seems that both are present in the State. The single record for *P. lecontei*, first reported by Hagen (1870) and later cited by Brimley (1938: 503), "Beaufort", is certainly erroneous. The range of *P. lecontei* is apparently restricted to southern Alabama and Mississippi; furthermore, Faxon examined Hagen's specimens from Beaufort and stated that they were *"C. blandingii"* [= *P. blandingii*]. *Procambarus pearsei* has been known previously only from the type locality in Cumberland County, North Carolina.

The new subspecies described here is known from three localities in the eastern part of the State, one in Craven County and the other two in Duplin.

Procambus pearsei pearsei (Creaser)

Cambarus pearsei Creaser, 1934. Occ. Pap. Mus. Zool., Univ. Mich., (285): 1–4, 3 figs.
Procambarus pearsei Hobbs, 1942. Amer. Midl. Nat., **28** (2): 342 (by implication).

Diagnosis.—Rostrum with or without marginal spines and concave or subplane above; postorbital ridges distinct and with or without a cephalic tubercle; suborbital angle obtuse; with or without a single lateral moderately well developed tubercle on each side of carapace; areola 3.7 to 8.1 times longer than broad and constituting 30.0 to 36.1 per cent of entire length of carapace. In male, ischiopodites of third and fourth pereiopods with simple hooks. Palm of chela not bearded but with a row of 9 to 11 tubercles. First pleopod of first form male with cephalodistal margin lacking a distinct hump and distal portion of appendage directed at an angle of 75° to 90° to main shaft of pleopod; mesial process non-corneous and directed caudolaterad; caudal process corneous, with distal extremity emarginate and directed caudolaterad; central projection corneous, beak-like, lying between caudal and cephalic processes and directed caudolaterad. Annulus ventralis with a subcircular median elevation bearing the dextrally tilted *S*-shaped sinus; cephalic wall emarginate near median line.

Type Locality.—Pond and ditch on Highway No. 22, south of Fayetteville, Cumberland Co., North Carolina.

Range.—In the collection of the senior author there are 185 specimens of this subspecies from the following: North Carolina—Bladen, Brunswick, Columbus, Johnson, Pitt, and Robeson counties; South Carolina—Horry County.

Procambarus p. pearsei is a secondary burrower (Hobbs, 1942: 20) frequenting ditches, borrow pits, temporary bodies of standing water, and small sluggish streams. During dry seasons this crayfish digs simple burrows that may reach depths greater than three feet. Since on several occasions a first form male and a female were found together in a burrow, it seems probable that mating takes place, at least in part, in burrows, as is the case in many other crayfishes.

Color Notes.—Dorsal portion of carapace and abdomen dark brown, the former bearing a narrow median longitudinal band of light grayish tan. Lateral portion of carapace cephalic to cervical groove dark brown above and bright pink below level of suborbital angle. Branchiostegites dark brown above fading to pinkish tan, and cream colored along lower margin; pinkish tan area brilliantly marked with splotches of cherry-red, and extreme cephaloventral portion, below cervical groove, brownish pink. Epimera and lateral portions of abdominal terga tannish pink with a dark brown longitudinal band along bases of the epimera. Chela dark brown above as is lower inner portion of palm; remainder of lower portion straw colored to cream. Other pereiopods greenish with dark markings at the joints. These notes were made on a first form male collected

[1] Contribution from the Mountain Lake Biological Station and the Samuel Miller Biological Laboratories, University of Virginia, Charlottesville.

[2] Department of Biology, University of Virginia, Charlottesville, and 1116 East Main Street, Danville, Virginia.

3.3 mi. S. of Elizabethtown, Bladen County, North Carolina.

VARIATIONS.—This species shows few variations in the characters which distinguish it from other crayfishes. The young often bear marginal spines on the rostrum, but the only variation noted that appears to be correlated with a restricted portion of the range is in the first pleopod. In specimens from the Neuse Drainage System in Johnston and Pitt counties (here considered to be "intergrades"), the tip of the mesial process of the first pleopod of the first form male is bent mesially and there is a distinct hump on the cephalodistal portion of the appendage—both characteristics of the new subspecies—whereas, in specimens from the more southern localities (typical *pearsei pearsei*) the mesial process is directed caudolaterally from base to apex and the cephalodistal margin of the appendage is evenly rounded. The caudal process of the pleopod in specimens from the Neuse Drainage System is directed caudolaterally and the distal portion of the appendage is strongly recurved as in the typical subspecies. The annulus ventralis is markedly uniform throughout the range. In specimens from one locality in Bladen County and those from Brunswick County the areola is somewhat broader (range of 3.7 to 5.8 times longer than broad—average 4.7) than elsewhere in the range. The corresponding averages in specimens from Columbus, Pitt, and Johnston counties, North Carolina and Horry County, South Carolina, are 6.0, 7.1, 6.9 and 6.1.

Procambarus pearsei plumimanus subsp. nov.[3]

DIAGNOSIS.—Rostrum with or without marginal spines and concave above; postorbital ridges well-defined and with a cephalic spine or tubercle; suborbital angle obtuse or absent; a single lateral spine or tubercle on each side of carapace; areola 5.0 to 7.3 times longer than broad and constituting 30.5 to 34.7 per cent of entire length of carapace. Simple hooks present on ischiopodites of third and fourth pereiopods in male. Palm of chela not bearded but with a row of 10 or 11 tubercles. First pleopod of first form male with a slight shoulder on cephalic margin at base of distal fourth and distal portion of appendage directed at about a 50° angle to main shaft of pleopod; mesial process non-corneous, slender, and directed caudodistad with its tip extending mesially; cephalic process, lying wholly mesiad of central projection, corneous, and directed caudodistad and slightly laterad; caudal process corneous with distal extremity entire, forming a compressed plate along caudolateral margin of distal portion of appendage and directed caudodistad; central projection in the form of a thin corneous plate forming with the caudal process an arc-like ridge along caudal and lateral margins. Annulus ventralis with a subcircular median elevation bearing the dextrally tilted S-shaped sinus; cephalic wall not deeply dissected near median line.

HOLOTYPIC MALE, FORM I.—Body subovate, compressed laterally; abdomen slightly longer than carapace (28.1 and 26.1 mm.). Height slightly greater than width in region of caudodorsal margin of cervical groove; greatest width of carapace slightly caudad of caudodorsal margin of cervical groove.

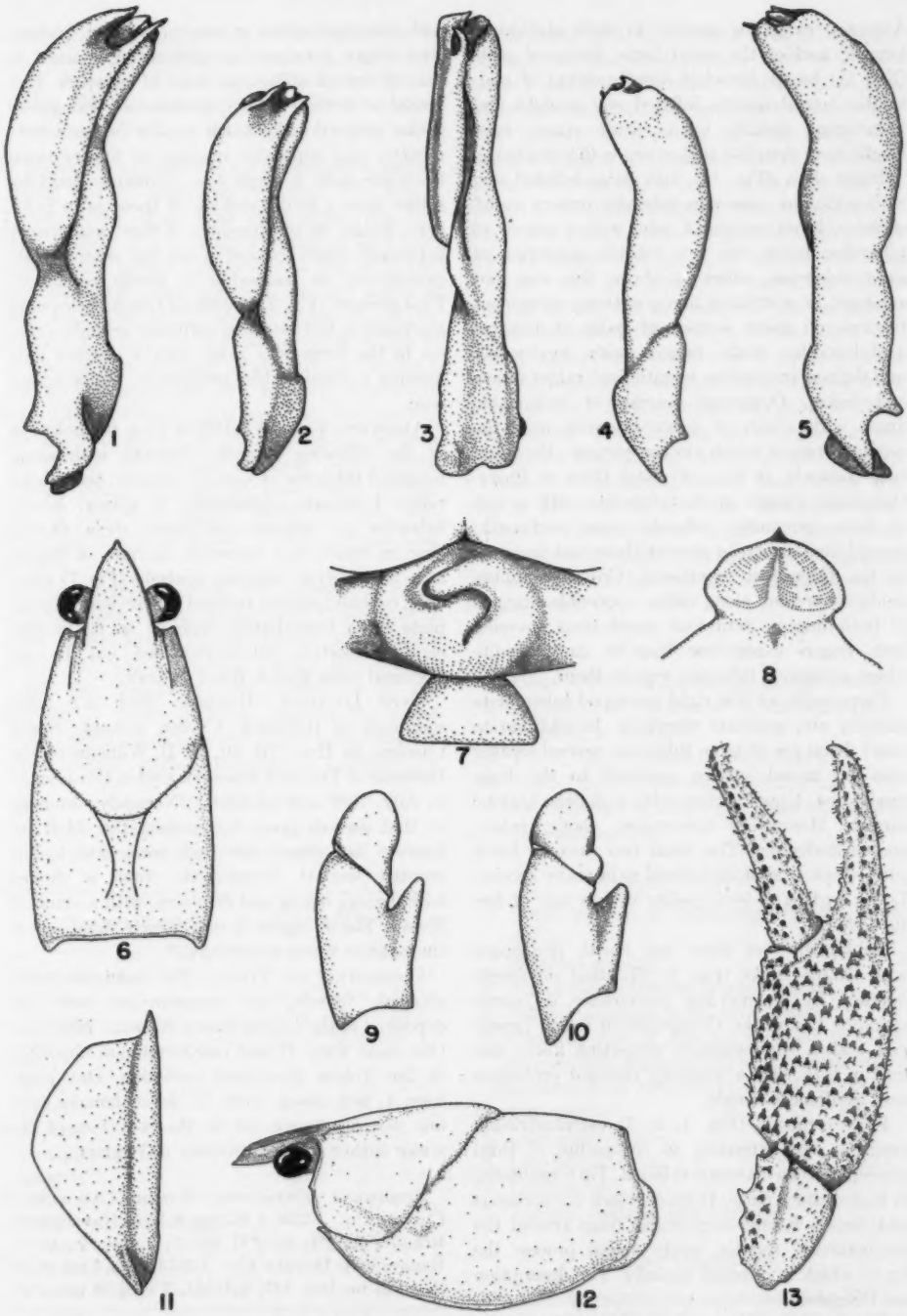
Areola about 5.5 times longer than broad with three punctations in narrowest part. Cephalic section of carapace about 2.1 times as long as areola (length of areola about 31.8 per cent of entire length of carapace).

Rostrum without marginal spines; margins slightly elevated but not thickened, and converging to small indistinctly delimited acumen. Upper surface subplane with scattered punctations and a row of punctations mesial to margins. No carina present. Postorbital angle almost obsolete, obtuse. Branchiostegal spine well developed, acute. Lateral surface of carapace granulate and a strong lateral tubercle present on each side just caudal to cervical groove. Dorsal surface of carapace punctate. Cephalic section of telson with two spines in each caudolateral angle.

Epistome (Fig. 8) broader than long with a triangular elevation (ventrally) along median line; cephalic border with a median projection.

[3] *pluma*: L., a small, soft feather; *manus*: L., hand; referring to the chela studded with plumose setae.

FIG. 1–13. *Procambarus pearsei plumimanus*, subsp. nov. Fig. 1. Mesial view of first pleopod of first form male. Fig. 2. Mesial view of first pleopod of second form male. Fig. 3. Caudal view of first pleopod of first form male. Fig. 4. Lateral view of first pleopod of second form male. Fig. 5. Lateral view of first pleopod of first form male. Fig. 6. Dorsal view of carapace. Fig. 7. Annulus ventralis. Fig. 8. Epistome. Figs. 9 and 10. Basipodites and ischiopodites of third and fourth pereiopods. Fig. 11. Antennal scale. Fig. 12. Lateral view of carapace. Fig. 13. Distal podomeres of cheliped of first form male.



FIGS. 1–13

Antennae extending caudad to sixth abdominal tergum, and of the usual form. Antennal scale (Fig. 11) broad; broadest slightly distad of midlength; lateral portion inflated and straight and terminating distally in an acute spine; total length more than half that of areola (6.0–8.3 mm.).

Right chela (Fig. 13) with palm inflated and with setiferous squamous tubercles present on all surfaces. Inner margin of palm with a row of 10 tubercles; below this row another consisting of eight tubercles; tubercles above this row not arranged in a striking linear pattern; prominent tubercle on lower surface of palm at base of dactylopodite. Both fingers with moderately well-defined submedian longitudinal ridges above and below. Opposable margin of immovable finger with a row of seven tubercles only the proximal two of which are conspicuous; the usual large tubercle at base of distal third of finger. Opposable margin of dactylopodite with a row of three prominent tubercles near midlength; several small tubercles present above and proximal to the three just mentioned. Crowded minute denticles present along entire opposable margins of both fingers. Setiferous punctations covering both fingers except for base of dactylopodite where squamous tubercles replace them.

Carpopodite of first right pereiopod tuberculate mesially and punctate elsewhere. In addition to usual distal arc of large tubercles, several smaller ones on mesial surface proximal to the large mesial one. Upper surface with a shallow oblique furrow. Meropodite tuberculate above, below, and mesiodistally. The usual two rows on lower surface not so sharply defined as in many species. Lower surface of ischiopodite with a row of five tubercles.

Ischiopodites of third and fourth pereiopods with simple hooks (Fig. 9, 10), that on fourth opposed by a knob-like prominence on corresponding basipodite. Coxopodite of fourth pereiopod with a caudomesially projecting knob, and that of fifth with a ventrally directed projection near caudomesial angle.

Fifth pleopods (Fig. 1, 3, 5) asymmetrically arranged and extending to coxopodite of third pereiopod when abdomen is flexed. Tip terminating in four distinct parts, three of which are corneous and form a lateral semicircular ridge around the non-corneous, slender, acute mesial process the tip of which is directed mesially. For description see Diagnosis.

MORPHOTYPIC MALE, FORM II.—Differs from the holotype in the following respects: rostrum with marginal spines at base of acumen; postorbital ridges terminate cephalically in spines; a pair of lateral spines on sides of carapace just caudal to cervical groove; epistome subtriangular; chelae proportionally much smaller (see measurements); and opposable margins of fingers more blade-like with a *single* row of minute denticles rather than a broad cushion of them as in holotype; hooks on ischiopodites of third and fourth pereiopods much reduced in size and no opposable prominence on basipodite of fourth pereiopod. First pleopod (Fig. 2, 4) with all terminal elements represented, however, non-corneous; cephalic process in the form of a ridge with a distinct lobe forming a shoulder-like prominence at its lateral base.

ALLOTYPIC FEMALE.—Differs from the holotype in the following respects: rostrum with small marginal tubercles at base of acumen; postorbital ridges terminate cephalically in spines; lateral tubercles on carapace spiniform; chela shorter than in males, and opposable margins of fingers as in morphotype. Annulus ventralis (Fig. 7) movable; cephalic portion covered by the large sternal plate lying immediately cephalic to it; median elevated portion subcircular and cut by an *S*-shaped sinus that is tilted dextrally.

TYPE LOCALITY.—Roadside ditch 2.2 miles southeast of Havelock, Craven County, North Carolina on Hwy. 70. Dr. A. B. Williams of the Institute of Fisheries Research visited this locality in July, 1957 and reported, "Normally, drainage in that area is poor. I examined the ditch for burrows but found few and none that looked recently worked. Usually the ditch is choked with *Juncus repens* and at places, with a stand of *Typha*. The *J. repens* is now largely dried out in the patches where it occurred."

DISPOSITION OF TYPES.—The holotypic male, allotypic female, and morphotypic male are deposited in the United States National Museum.[4] One male, form II and one female are deposited in the Tulane Zoological collection. One male, form I, two males, form II, seven females, and one juvenile female are in the collection of the senior author at the University of Virginia.

SPECIMENS EXAMINED.—NORTH CAROLINA—CRAVEN CO.: 3-2556-1, 2.2 mi. S.E. of Havelock on Rte. 70, 2♂♂I, 4♂♂II, 9♀♀, 1 juv. ♀, E. C. Raney, coll. DUPLIN CO.: 4-2355-2a, 2.4 mi. S. of Rosehill on Rte. 117, 3♂♂II, 3♀♀, 10 juv.♂♂,

[4] U.S.N.M. nos. 100940, 100941, and 100942, respectively.

9 juv. ♀♀, H. H. H., coll.; 4-1756-10a, 4.0 mi. E. of Kenansville on Rte. 24, 2♂♂I, 1♂II, 2♀♀, E. T. Hall and H. H. H., coll. These localities lie within the drainage systems of the Neuse and Cape Fear rivers.

VARIATIONS.—There is relatively little variation among the 46 specimens available. The largest specimens are those from Kenansville, one first form male having a carapace length of 35.8 mm. whereas the largest specimen from the other two localities is the allotype with a carapace length of 26.6 mm. The length of the areola constitutes from 30.5 to 34.7 per cent of the entire length of the carapace, those from Duplin County having a slightly higher average, 32.7 per cent, than those from Craven County, 31.7 per cent. The ratio of the length to the width of the areola ranges from 5.0 to 7.3, the average of those from Duplin County being 6.0, and of those from Craven County, 5.4. The smaller specimens have well developed marginal spines on the rostrum near its apex.

RELATIONSHIPS.—These two subspecies probably have their closest affinities with *P. planirostris* Penn (1953: 71), *P. hybus* Hobbs and Walton (1957: 39), and *P. mancus* Hobbs and Walton (1957: 44). Regarding their relationship to one another, it seems probable that in the lower coastal plain the ranges of the two subspecies are not in contact; perhaps this may be explained by the presence of a well drained area in Sampson County and the northwestern part of Pender County and it is not unlikely that the Cape Fear River itself might act as a barrier in the Brunswick-New Hanover county area. It seems likely that some exchange of genes has occurred or is occurring in the Johnston-Pitt county region. Too little is known of the distribution of either subspecies in the surrounding counties to postulate where this gene exchange took place, if indeed such has occurred. The two subspecies cannot be distinguished except on the basis of the secondary sexual characteristics; here, however, there are several distinct differences, and it seems unlikely that the admixture of these in specimens from Johnston and Pitt counties has resulted from convergence; also there is no evidence for an assumption that the populations in these counties represent the ancestral stock from which the typical members of the two subspecies have diverged. Because these forms are somewhat intermediate in their anatomy between *pearsei* and *plumimanus*, the two are here considered to be subspecies.

TABLE 1

Some measurements of type specimens of Procambarus pearsei plumimanus *(in mm.)*

Structure measured	Holotype	Allotype	Morphotype
Carapace:			
Height	13.0	12.6	11.1
Width	12.6	12.5	10.7
Length	26.1	26.6	23.4
Areola:			
Width	1.5	1.5	1.4
Length	8.3	8.3	7.2
Rostrum:			
Width	4.8	4.3	4.3
Length	7.0	7.2	6.4
Chela:			
Length of inner margin of palm	9.0	5.0	4.7
Width of palm	7.0	4.6	4.2
Length of outer margin of chela	21.1	13.4	12.3
Length of dactyl	10.9	8.0	7.0

Typical *pearsei*

1. Distal portion of appendage directed caudad.
2. Cephalodistal portion of appendage evenly rounded; no shoulder.
3. Tip of mesial process directed laterad.
4. Caudal process directed caudolaterad.
5. Cephalic wall of annulus ventralis emarginate near median line.

Typical *plumimanus*

1. Distal portion of appendage directed more distad.
2. Shoulder present.
3. Tip of mesial process directed mesiad.
4. Caudal process directed caudodistad.
5. Cephalic wall of annulus ventralis with no conspicuous emargination near median line.

Specimens from Johnston and Pitt Counties

1. Like *pearsei*
2. Like *plumimanus*
3. Like *plumimanus*
4. Like *pearsei*
5. Annulus ventralis like *pearsei*

ACKNOWLEDGMENTS

We wish to express our appreciation to Dr. E. C. Raney, Dr. W. R. West, and Mr. E. T. Hall for their assistance in adding to our knowledge of the

distribution of these two crayfishes and to Dr. A. B. Williams for his observations on the type locality.

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A TAXONOMIC CHANGE IN THE SUBFAMILY TOMOCERINAE (ORDER COLLEMBOLA)

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It is inevitable that taxonomic difficulties will arise within any group of organisms from time to time. When dealing with a group as vast as the Class Insecta, the number of taxonomic discrepancies is proportionately great. It is possible to follow this singular line of reasoning even further and state that the status of systematics is far from stable in groups of insects which have been exposed to comparatively little research in contrast to other well-worked categories. Such is the case with the Order Collembola, a group of wingless insects, which have received relatively little attention in many parts of the world.

Aside from short papers, few monographs have been published on the North American collembolan fauna. Despite the tremendous numbers of Collembola extant in many areas, few regions have been intensively examined. Guthrie's monograph published in 1903, Mills' contribution on Iowa Collembola (1934), and Maynard's impressive work which appeared in 1951, are the only definitive works on the collembolan fauna for any extensive geographic regions. As a consequence, insect surveys and ecological studies on restricted groups of this order in other parts of North America will undoubtedly result in a number of taxonomic revisions.

A problem of this nature has recently become apparent as a result of an ecological investigation of the Subfamily Tomocerinae in the Piedmont region of North Carolina. The Genus *Tomocerus* contains a number of trivial categories; Mills lists three species for Iowa, Maynard's monograph cites five species for New York. One species, *Tomocerus flavescens*, contains a number of infraspecific forms and subsequently it is designated as a polytypic species. Maynard lists four subspecies of *T. flavescens* for the state of New York.

According to modern systematics, any two infraspecific forms of a single species are potentially capable of interbreeding. Consequently, in order to maintain the subspecies there must be some means of isolation. Mayr, Linsley and Usinger (p. 30) make the situation quite clear in these words: "Not more than one subspecies of any one polytypic species can exist in breeding condition in any one area. Adjacent subspecies interbreed or are potentially capable of doing so if separated by extrinsic barriers."

During an annual collection cycle the two infraspecific forms of *Tomocerus*, *T. flavescens americanus* and *T. flavescens separatus*, were found coexisting in the same quadrats and in the same stratum of the litter in six of the eight wooded localities that were studied. If two populations of infraspecific forms are not geographically isolated, then it would be expected that there would be a reproductive, ecological, diurnal or genetic isolation. As a result of field study and careful laboratory observation, none of the above mechanisms of isolation appeared to be in operation. It was evident that reproduction occurred during the same season of the year, as eggs and young of both *T. flavescens americanus* and *T. flavescens separatus* were found at the same time of year. If interbreeding occurred between the two 'subspecies' at any time, intermediate forms (at least sterile hybrids) would have been expected, but this situation was never encountered during the entire investigation.

It is proposed that these two infraspecific forms be placed in separate species on the basis of this evidence. *Tomocerus flavescens americanus* should be returned to its original taxonomic category, *Tomocerus americanus* Schött 1896, and *Tomocerus flavescens separatus* would remain in its present taxonomic position unless further study should justify its removal from the species, *T. flavescens*, and the subsequent elevation of this form to a distinct trivial category.

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STUDIES ON THE INFLUENCE OF A PRIOR INFECTION WITH *ANCYLOSTOMA CANINUM* ON THE ESTABLISHMENT AND MAINTENANCE OF *TRICHINELLA SPIRALIS* IN MICE[1]

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The presence of two parasitic infections in one host has a varied effect upon the establishment and maintenance of the separate parasites. In some instances, one infection produces effects that tend to inhibit the other infection, while in other situations the effects tend to favor the development of the other infection.

In certain dual infections, the time and severity of the second infection appear to influence markedly the course of the first infection. For example, Yoeli (1956) found that *Plasmodium berghei* infections given to voles shortly before or after a *Schistosoma mansoni* infection showed a high proportion of severe infections produced by the former. However, when the *P. berghei* infection was given as late as 4 to 7 weeks after *S. mansoni*, the malaria infection showed mild effects, low parasitemia, and no fatalities. It was postulated that the lymphoid-macrophage system, alerted by the schistosome infection, had increased phagocytic powers and was, therefore, able to affect the course of the malaria infection.

Cox (1952) showed that a previous infection with *Ancylostoma caninum* produced effects that inhibited the development of *Trichinella spiralis*. It should be noted that only certain time intervals, selected arbitrarily, were used in demonstrating this effect. On the basis of these findings, the author suggested that the effect was probably caused by the local inflammation resulting from the *A. caninum* infection. It is known that cellular inflammation in the intestinal tissues of mice is not evident until 24 hours after an initial *A. caninum* infection, after which it requires many days to run its course (Kerr, 1936). Therefore, if the inflammatory response to *A. caninum* is involved in this effect, the scheduling of the *T. spiralis* infection becomes an important consideration.

The present study was designed to determine the importance of elapsed time between the infections in altering a *T. spiralis* infection in mice given a prior infection with *A. caninum*. Also, an attempt was made to determine the portion of the small intestine where the reduction of the *T. spiralis* population is most evident. With these determinations, it was hoped that the mechanism(s) of the immune response to *T. spiralis* could be understood more fully.

[1] A portion of a Dissertation submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the School of Public Health.

Materials and Methods

The animals used in this study were a strain of Swiss white mice that have been maintained by this Department for a number of years. Equal numbers of males and females, 16 weeks old at the beginning of each experiment, were used.

The procedures for standardization and infection with the *Trichinella spiralis* larvae and the isolation and recovery of the adult worms from the small intestine were the same as those described by Larsh and Kent (1949) and others, with a few minor changes.

Ancylostoma caninum larvae were obtained from an infection maintained in a mongrel dog. Charcoal cultures were made with the feces containing eggs. The cultures were incubated for 7 days at room temperature and the infective larvae were collected in a Baermann apparatus. The larvae were suspended in tap water and standardized for infection by methods similar to those used for *T. spiralis*.

The criterion for measurement of the effect produced by *A. caninum* was the number of adult *T. spiralis* worms harbored in the small intestine at 3, 5, 7, 9, or 11 days after infection. The location of the action(s) causing the worm elimination was determined by dividing the small intestine into quarter-sections, and counting the number of adult worms harbored in each section.

The data from the experiments were analyzed statistically by the analysis of variance test. The calculations and tables of these analyses are on file, Department of Parasitology, University of North Carolina.

EXPERIMENTAL PROCEDURES AND RESULTS

SERIES ONE. *Design.*—The purpose of the seven experiments of the first series was to determine the effect of different time intervals between the administration of the *A. caninum* and *T. spiralis* infections on the establishment and maintenance of the *T. spiralis* infection. All experiments were identical except that a different time interval was used between the administration of the two agents for each experiment. The infections were given simultaneously, or the *A. caninum* infection was given at 12, 24, 48, 96, 144, or 192-hour intervals prior to the *T. spiralis* infection.

For each experiment, the mice were divided into four major groups with 8 mice in each group. Groups I and III (experimentals) received 800 *A. caninum* larvae *per os* while Groups II and IV (controls) received no treatment. Appropriate viability controls were included in all instances. At the designated time interval after the *A. caninum* infection, all animals were infected with *T. spiralis*. Groups I and II received an infection with 200 *T. spiralis* larvae and Groups III and IV received 400 larvae. Necropsy was performed 7 days after the infection with *T. spiralis* and the number of adult *T. spiralis* worms harbored in the entire small intestine was counted.

Results.—The average number of *T. spiralis* adults recovered from the groups of experimental and control mice for each experiment is shown in Table 1. Percentages are given as the ratio of the number of adults recovered to the number of larvae administered in the infecting dose.

When the infections with *A. caninum* and *T. spiralis* were given simultaneously, the *A. caninum* infection had no significant effect on the establishment of the *T. spiralis* infection. From an infection with 200 larvae, an average of 127.8 adult *T. spiralis* worms was recovered from the small intestine of the animals that had received both infections (Group I), while an average of 140.9 worms was recovered from the controls (Group II). Similarly, the experimental animals that received 400 larvae (Group III) harbored an average of 314.4 worms and the controls (Group IV) harbored an average of 330.8 adult worms.

An interval of 12 hours between the infections gave similar results. The experimental and control groups that received 200 *T. spiralis* larvae showed an average recovery of 125.2 worms and 136.1 worms, respectively, while those that received 400 larvae harbored an average of 299.4 worms and 313.9 worms, respectively.

On the other hand, when intervals of 24 or 48 hours elapsed between infections, the *A. caninum* infection had a significant effect in reducing the number of *T. spiralis*. With the 24-hour interval, an average of 116.1 worms was recovered from the experimentals receiving 200 larvae (Group I), while the controls (Group II) harbored an average of 175.2 worms. The experimentals that received 400 larvae (Group III) harbored an average of 240.6 worms and the controls (Group IV) harbored 340.6 worms. Using the 48-hour interval, an average of 127.6 worms was recovered from the experimentals receiving 200 larvae (Group I), while the controls (Group II) harbored an average of 162.5 worms. The experimentals that received 400 larvae (Group III) harbored an average of 250.6 worms and the controls (Group IV) harbored an average of 348.5 worms.

The last 3 experiments of the series, using intervals of 96, 144, and 192 hours, were carried out to obtain complete data concerning the effect produced

TABLE 1

Average worm recoveries from experimental and control groups (8 mice per group) 7 days after receiving 200 and 400 T. spiralis *larvae at designated time intervals after infection with 800* A. caninum

Interval Between Infections (hours)	Infection with 200 *T. spiralis*			Infection with 400 *T. spiralis*		
	Group I Experimental (per cent)	Group II Control (per cent)	Mean Differences	Group III Experimental (per cent)	Group IV Control (per cent)	Mean Differences
0	127.8 (63.9)	140.9 (70.4)	13.1	314.4 (78.6)	330.8 (82.7)	16.4
12	125.2 (62.6)	136.1 (68.0)	10.9	299.4 (74.8)	313.9 (78.5)	14.5
24	116.1 (58.0)	175.2 (87.6)	59.1	240.6 (60.2)	340.6 (85.2)	100.0
48	127.6 (63.8)	162.5 (81.2)	34.9	250.6 (62.6)	348.5 (87.1)	97.9
96	166.2 (83.1)	174.5 (87.2)	8.3	305.0 (76.2)	339.2 (84.8)	34.2
144	158.1 (79.0)	169.5 (84.8)	11.4	331.4 (82.8)	337.0 (84.2)	5.6
192	175.0 (87.5)	175.4 (87.7)	0.4	260.8 (65.2)	278.8 (69.7)	18.0

by a previous *A. caninum* infection. As shown in Table 1, the differences between the mean recoveries for the experimental and control groups receiving 200 *T. spiralis* were 8.3 worms at 96 hours, 11.4 worms at 144 hours, and 0.4 worms at the 192-hour interval. The mean differences between the groups receiving 400 larvae were 34.2 worms at 96 hours, 5.6 worms at 144 hours, and 18.0 worms at the 192-hour interval. Thus, infection with *A. caninum* given 96, 144, or 192 hours prior to infection with *T. spiralis* had no significant effect on the *T. spiralis* infection.

To obtain an integrated statistical analysis involving all observations, a weighted analysis of variance of mean differences was carried out. This was necessary because variation was naturally greater in the groups that had received 400 *T. spiralis* larvae than in the groups that had received only 200 larvae.

The analysis showed that the effect of different time intervals between infections is statistically significant (1% level). Also, it showed that the two dosages of *T. spiralis* had a significantly different effect (5% level), i.e., the mean differences between the experimental and control groups of mice infected with 400 larvae were significantly greater than the differences from mice infected with only 200 larvae.

SERIES TWO. *Design.*—The demonstration in Series One of a significant reduction in the number of *T. spiralis* harbored in the small intestine of mice given an infection with *A. caninum* 24 or 48 hours prior to the *T. spiralis* infection encouraged the design of additional experiments. These experiments were carried out to determine: (1) the interval required after the *T. spiralis* infection for the occurrence of the significant worm reduction in the small intestine, (2) the relative location of the action(s) within the small intestine that causes the elimination of the *T. spiralis* adults, and (3) the effect of an *A. caninum* infection on the acquired immunity against *T. spiralis* resulting from one stimulating infection. Because of the large number of animals involved, and the plan to make individual worm counts for each quarter-section of each intestine, 2 experiments were necessary. The experiments were identical except that different dates of necropsy were used in each instance. One experiment incorporated necropsy periods of 3 and 5 days, while the other experiment used periods of 7, 9, and 11 days.

The mice were divided into 3 major groups (Tables 2 and 3), and viability controls were included. The animals of Group I were given a stimulating infection with 200 *T. spiralis* larvae. Twenty-one days later, the animals of Groups I and II were given an infection with 800 *A. caninum* larvae. Twenty-four hours after the *A. caninum* infection, all animals (Groups I, II, and III) were given a challenging infection with 400 *T. spiralis* larvae. On days 3, 5, 7, 9, and 11 after the second infection with *T. spiralis*, 8 animals from each group were sacrificed and the number of *T. spiralis* adults in each quarter-section of the small intestine was counted.

Results.—The numbers of adult *T. spiralis* worms recovered from the quarter-sections of the small intestine on the third, fifth, seventh, ninth, and eleventh days after infection are recorded in Tables 2 and 3.

The number of worms recovered from the entire small intestine of the controls (Group III) remained relatively constant throughout the entire period. On day 3, the average total recovery was slightly lower (172.1 worms) than the average recoveries for the remaining necropsy dates (206.6, 211.8, 209.2, and 202.6 worms). It is possible that some of the worms were too deeply embedded in the intestinal tissues to be recovered on the third day.

A significant reduction in the number of *T. spiralis* adults harbored in the entire small intestine had already occurred by the third day after infection in the animals of Group II that had received an infection with *A. caninum* 24 hours previously. Thus, the mean worm recoveries were similar for all necropsies (146.4, 155.2, 148.0, 147.4, and 139.5 worms). On the other hand, the animals of Group I that had received prior infections with both *T. spiralis* and *A. caninum* did not harbor significantly fewer worms until the fifth day after infection. On the third day, the total mean recovery was 209.5 worms, while the remaining necropsies showed mean recoveries of 148.0, 149.9, 146.6, and 151.1 worms, respectively.

The data from both experiments were combined and subjected to an analysis of variance. The analysis showed a significant variation between the different groups of mice. This indicated a significant reduction in the number of *T. spiralis* adults harbored in the entire small intestine of animals that had received a prior infection(s) with *T. spiralis* and/or *A. caninum*. Also, a highly significant variation between the worm recoveries from the different sections of the small intestine was indicated. By calculation, the

TABLE 2

The number of T. spiralis *adults recovered from each quarter-section of the small intestine three and five days after an infection with 400 larvae*

Days After Infection	Mouse	Group I Infected with 200 *T. spiralis* 22 Days Previously and 800 *A. caninum* 24 Hours Previously							Group II Infected with 800 *A. caninum* 24 Hours Previously							Group III No Treatment						
		Section of Intestine							Section of Intestine							Section of Intestine						
		1	2	1st Half	3	4	2nd half	Total	1	2	1st half	3	4	2nd half	Total	1	2	1st half	3	4	2nd half	Total
3	1	36	62	98	106	23	129	227	13	38	51	65	34	99	150	51	104	155	21	2	24	178
	2	39	54	93	82	28	110	203	29	55	84	65	12	77	161	46	87	133	36	6	42	175
	3	10	45	55	109	13	122	177	33	16	49	66	41	107	156	63	65	128	20	3	23	151
	4	13	90	103	85	11	96	199	40	83	123	40	17	57	180	36	65	101	47	4	51	152
	5	36	14	50	83	25	108	158	52	29	81	30	10	40	121	89	115	204	18	3	21	225
	6	37	86	123	74	18	92	215	81	45	126	37	12	49	175	67	70	137	49	8	57	194
	7	58	142	200	36	21	57	257	37	28	65	19	8	27	92	51	57	108	24	4	28	136
	8	20	71	91	121	28	149	240	65	33	98	26	12	38	136	41	86	127	38	1	39	166
	Mean	31.1	70.5	101.6	87.0	20.9	107.9	209.5	43.8	40.9	84.6	43.5	18.2	61.8	146.4	55.5	81.1	136.6	31.6	3.9	35.6	172.1
5	1	13	100	113	37	3	40	153	23	48	71	63	49	112	183	82	104	186	58	13	71	257
	2	4	13	17	97	39	136	153	24	23	47	22	73	95	142	41	110	151	70	11	81	232
	3	29	58	87	42	18	60	147	24	44	68	86	22	108	176	86	65	151	39	9	48	199
	4	24	37	61	102	28	130	191	40	37	77	87	35	122	199	48	88	136	44	1	45	181
	5	5	58	63	72	22	94	157	34	18	52	19	33	52	104	64	78	142	39	6	45	187
	6	19	21	40	34	16	50	90	6	31	37	70	27	97	134	94	104	198	24	2	26	224
	7	33	30	63	54	25	79	142	56	54	110	30	24	54	164	66	76	142	41	5	46	188
	8	14	47	61	68	22	90	151	29	19	48	39	53	92	140	78	72	150	33	2	35	185
	Mean	17.6	45.5	63.1	63.2	21.6	84.9	148.0	29.5	34.2	63.8	52.0	39.5	91.5	155.2	69.9	87.1	157.0	43.5	6.1	49.6	206.6

TABLE 3

The number of T. spiralis *adults recovered from each quarter-section of the small intestine seven, nine, and eleven days after an infection with 400 larvae*

Days After Infection	Mouse	Group I Infected with 200 *T. spiralis* 32 Days Previously and 800 *A. caninum* 24 Hours Previously							Group II Infected with 800 *A. caninum* 24 Hours Previously							Group III No Treatment						
		Section of Intestine							Section of Intestine							Section of Intestine						
		1	2	1st half	3	4	2nd half	Total	1	2	1st half	3	4	2nd half	Total	1	2	1st half	3	4	2nd half	Total
7	1	3	36	39	70	48	118	157	18	49	67	34	29	63	130	87	78	165	53	1	54	219
	2	23	70	93	48	17	65	158	15	61	76	79	11	90	166	54	69	123	44	4	48	171
	3	7	82	89	91	26	117	206	11	63	74	70	47	117	191	61	111	172	80	16	96	268
	4	13	63	76	38	25	63	139	15	55	70	63	36	99	169	33	74	107	83	10	93	200
	5	14	45	59	71	56	127	186	23	54	77	71	21	92	169	37	110	147	76	7	83	230
	6	9	38	47	56	23	79	126	29	42	71	29	20	49	120	65	79	144	22	1	23	167
	7	12	34	46	16	29	45	91	25	43	68	50	11	61	129	50	105	155	64	17	81	236
	8	5	11	16	64	56	120	136	20	25	45	48	17	65	110	31	73	104	83	16	99	203
	Mean	10.8	47.4	58.1	56.8	35.0	91.8	149.9	19.8	49.0	68.5	55.5	24.0	79.5	148.0	52.2	87.4	139.6	63.1	9.0	72.1	211.8
9	1	8	10	18	42	43	85	103	25	85	110	40	16	56	166	12	32	44	92	40	132	176
	2	6	40	46	82	36	118	164	14	23	37	60	40	100	137	12	49	61	95	44	139	200
	3	14	64	78	55	37	92	170	9	61	70	73	40	113	183	18	99	117	93	21	114	231
	4	25	63	88	61	40	101	189	3	16	19	69	43	112	131	15	46	61	67	109	176	237
	5	13	23	36	58	45	103	139	33	36	69	31	12	43	112	23	16	39	34	110	144	183
	6	23	23	46	54	19	73	119	31	32	63	38	18	56	119	42	90	132	103	22	125	257
	7	3	60	63	23	42	65	128	20	53	73	66	23	89	162	16	19	35	53	88	141	176
	8	8	15	23	90	48	138	161	14	63	77	61	31	92	169	12	43	55	76	83	159	214
	Mean	12.5	37.2	49.8	58.1	38.8	96.9	146.6	18.6	46.1	64.8	54.8	27.9	82.6	147.4	18.8	49.2	68.0	76.6	64.6	141.2	209.2
11	1	8	30	38	29	36	65	103	1	12	13	51	72	123	136	15	19	34	67	62	129	163
	2	11	34	45	39	36	75	120	10	26	36	75	45	120	156	22	72	94	79	36	115	209
	3	9	51	60	90	35	125	185	3	56	59	39	57	96	155	30	97	127	52	63	115	242
	4	15	85	110	35	28	63	163	5	42	47	43	47	90	137	19	65	84	108	33	141	225
	5	13	53	66	45	47	92	158	6	46	52	75	55	130	182	8	105	113	77	67	144	257
	6	5	25	30	82	34	116	146	12	24	36	39	17	56	92	17	45	62	36	70	106	168
	7	21	32	53	63	72	135	188	16	28	44	48	41	89	133	17	28	45	50	54	104	149
	8	9	40	49	66	31	97	146	13	17	30	64	31	95	125	21	65	86	67	55	122	208
	Mean	11.4	43.8	56.4	56.1	39.9	96.0	151.1	8.2	31.4	39.6	54.2	45.6	99.9	139.5	18.6	62.0	80.6	67.0	55.0	122.0	202.6

weighted mean recoveries for each section of the small intestine from all animals were determined as follows: section 1—24.1 worms, section 2—50.4 worms, section 3—54.8 worms, and section 4—25.7 worms.

Most important, the interaction between the groups, the day of necropsy, and the section of the intestine was highly significant. This indicated that the distribution of the adult worms in the intestine was different for the experimental and control mice at each necropsy. Therefore, to show the relative location of the adult worms within the intestine, the weighted mean recoveries from each section of the intestine from each group of mice on the different days of necropsy were determined (Table 4) and plotted in Figure 1.

On the third day after infection, the majority of the worms recovered from the controls (Group III) were found in the anterior sections of the intestine (weighted averages of 54.2 and 79.9 worms, with 30.7 and 3.6 worms in the posterior sections). However, by the ninth day after infection, large numbers were harbored in the posterior sections (weighted averages of 75.0 and 59.4 worms, with 18.0 and 45.6 worms in the anterior sections).

On the third day after infection, the animals that had received a previous infection with *A. caninum* (Group II) harbored in sections 1 through 4, respectively, weighted averages of 42.0 worms, 38.9 worms, 41.7 worms, and 16.8 worms. Therefore, at this time these mice had about the same number of worms in the anterior and posterior halves of the small intestine, whereas the controls (Group III) harbored about 4 times as many in the anterior half. By the fifth day after infection, the Group II mice had more worms in the posterior than in the anterior half. This was true for the remaining necropsy periods, being most striking 11 days after infection when the mean recoveries for sections 1 through 4, respectively, were 7.1, 29.8, 53.6, and 44.2 worms.

On the third day after infection, the experimentals that had received prior infections with both *T. spiralis* and *A. caninum* (Group I) harbored in sections 1 through 4, respectively, weighted averages of 29.3, 65.8, 86.6, and 20.3 worms. These results indicate that many of the worms were already situated in the posterior half of the intestine 3 days after infection. The average number of adult worms remaining in the anterior half of the intestine became smaller at each successive necropsy, until 11 days after infection the weighted mean recoveries for sections 1 through 4, respectively, were 10.9, 42.1, 54.3, and 39.1 worms.

Therefore, as indicated above, a shift in the *T. spiralis* population occurred within the small intestine of all animals. Even after the significant reduction in the total number of adult *T. spiralis* harbored in the entire small intestine, the number of worms found in the first quarter-section became relatively smaller at each successive necropsy, while the number in the fourth quarter-section became progressively larger (Fig. 1). These findings have immunologic significance which will be discussed later.

It is also interesting to note that, in animals of Group I, the elimination of most of the worms from the first quarter of the small intestine had taken place prior to the fifth day after infection,

TABLE 4

Weighted mean recoveries of* T. spiralis *adults from each quarter-section of the small intestine after an infection with 400 larvae*

Days After Infection	Group I Prior Infections with 200 *T. spiralis* at 22 Days and 800 *A. caninum* at 24 Hours				Group II Prior Infection with 800 *A. caninum* at 24 Hours				Group III No Treatment			
	Section of Intestine				Section of Intestine				Section of Intestine			
	1	2	3	4	1	2	3	4	1	2	3	4
3	29.3	65.8	86.6	20.3	42.0	38.9	41.7	16.8	54.2	79.9	30.7	3.6
5	15.9	42.0	59.1	20.1	31.2	33.1	48.7	38.2	68.7	86.5	42.4	5.3
7	9.9	44.6	51.0	33.5	19.2	48.3	56.0	22.7	51.1	86.5	61.0	7.0
9	11.7	33.9	56.2	38.8	17.0	43.4	53.9	26.5	18.0	45.6	75.0	59.4
11	0.9	42.1	54.3	39.1	7.1	29.8	53.6	44.2	18.2	58.4	65.0	54.5

* These are the squares of the means of the square roots and are, in effect, means of the original recoveries appropriately weighted with respect to their relative errors.

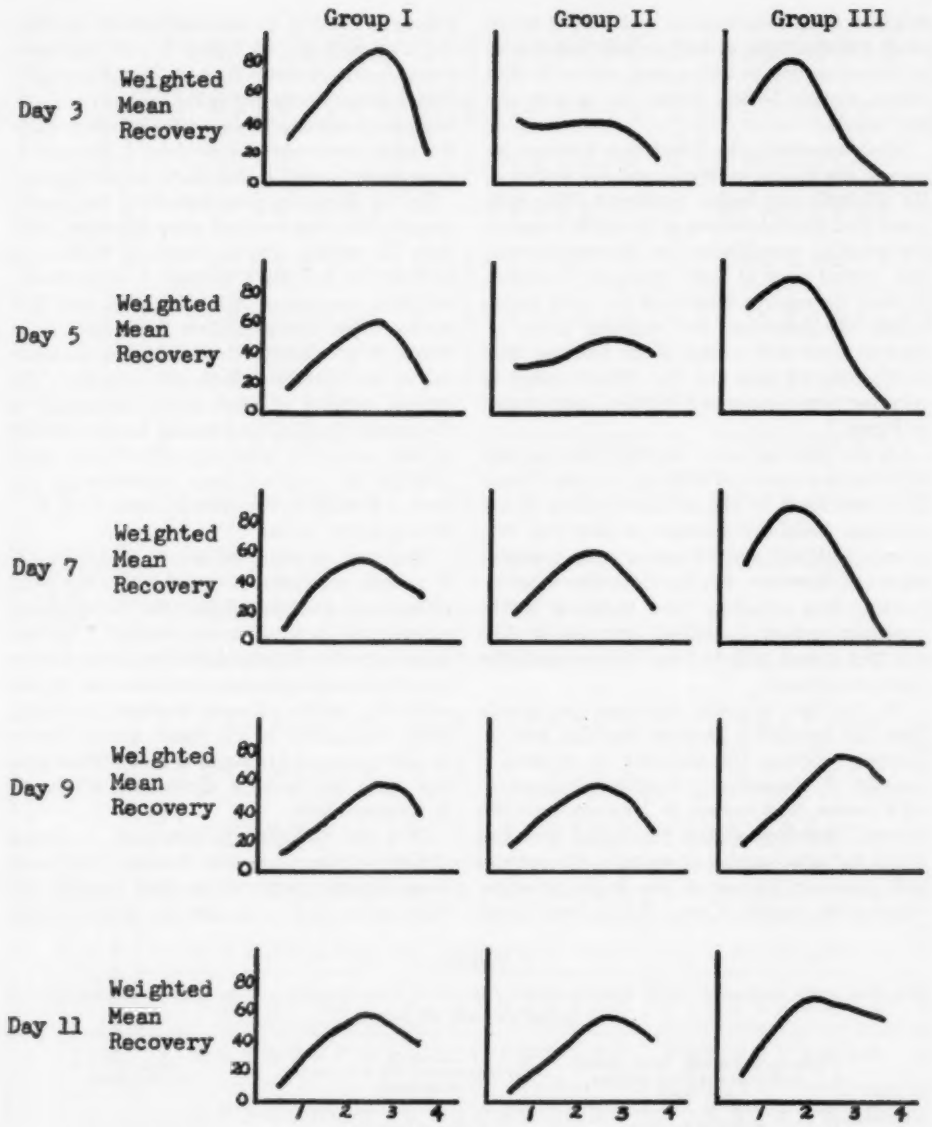

FIG. 1. The distribution of adult *T. spiralis* in the small intestine of mice after an infection with 400 larvae. Groups I and II had received a prior infection(s) with *T. spiralis* and/or *A. caninum*.

even though a significant reduction in the total worm recovery was not evident until 5 days after infection. On days 3, 5, and 7, the weighted mean recoveries from the first quarter of the small intestine were 29.3, 15.9, and 9.9 worms compared to mean recoveries of 42.0, 31.2, and 19.2 worms from animals of Group II (Table 4). This evidence indicated that a greater reduction in the number of *T. spiralis* adults occurred in the first quarter of the intestine at an earlier date in these animals

that had received earlier infections with *A. caninum* and *T. spiralis*, than in the animals that had received only the *A. caninum* infection given 24 hours previously.

DISCUSSION

The results from the experiments of Series One indicate that the time interval between infections with *Ancylostoma caninum* and *Trichinella spiralis* had an important role in eliminating adult worms from the *T. spiralis* infection. In these experiments, a significant reduction in the number of adult *T. spiralis* was evident only when the *A. caninum* infection was administered 24 or 48 hours before the infection with *T. spiralis* (Table 1). The adult *T. spiralis* population was not reduced when the interval between the infections was shorter than 24 hours or longer than 48 hours. These findings are not in total agreement with those of Cox (1952) who showed a significant reduction in the *T. spiralis* worm burden by an infection with *A. caninum* given 10 days earlier. The 10-day interval was not used in the present study, but intervals of 6 and 8 days failed to bring about a significant reduction in the number of *T. spiralis* adults. Also, the trend analysis gave no indication that intervals longer than 8 days would bring about a significant reduction. There is no explanation for the different results obtained.

The mechanism(s) involved in the elimination of adult *T. spiralis* by a prior *A. caninum* infection was not studied. However, on the basis of previous work, showing the pattern of the cellular response in the intestine of mice after an initial infection with *A. caninum*, it would appear that a non-specific inflammatory response is involved. There is no evidence of a cellular response 12 hours after infection, but neutrophilic infiltration is evident at 24 hours (Kerr, 1936). The cellular response appears to reach a zenith at 48 hours, after which it diminishes gradually until about 120 hours when it can no longer be demonstrated. It would appear, therefore, that when the inflammatory response to *A. caninum* is in its most acute phase (24–48 hours after infection), it has a deleterious effect upon the maintenance of *T. spiralis* in the same general area of the small intestine. Whether the cellular components act directly upon the adults of *T. spiralis* or merely have an indirect effect, by creating an unfavorable habitat, is not known. In any event, it is clear from histopathologic observations in mice infected only with *T. spiralis* (Larsh and Race, 1954; Larsh *et al.*, 1956) that specific inflammatory factors, alone or in combination with specific humoral factors, could not have been responsible for the reductions noted. Following an initial *T. spiralis* infection there is no evidence of an inflammatory response before 4 days and the zenith of the response is not reached until 8 days after infection. It is not until a few days after this, viz. 11–14 days after infection, that a significant number of worms is expelled from the anterior half of the small intestine (Larsh *et al.*, 1952).

The experiments of Series Two provided further evidence for the hypothesis that the reduction in the number of *T. spiralis* adults is brought about by the non-specific inflammation resulting from the previous *A. caninum* infection. As shown in Tables 2 and 3, the number of adults of *T. spiralis* in the controls (Group III) remained relatively constant for 11 days after the one infection. As indicated above, a significant reduction in adults does not occur until between 11–14 days after initial infection (Larsh *et al.*, *loc. cit.*; and others). On the other hand, both groups of mice that had received a prior infection with *A. caninum* showed a significant reduction in the number of *T. spiralis* soon after the challenging infection. This reduction had occurred by the third day in the animals that had received only a prior infection with *A. caninum* (Group II) and by the fifth day after infection in those that had received previous infections with both *T. spiralis* and *A. caninum* (Group I). It seems clear, therefore, that, in both cases, the previous *A. caninum* infection played a role in the reductions.

The earlier reduction in the Group II mice that had received only *A. caninum* prior to the challenging *T. spiralis* infection would, at first glance, seem to suggest that the stimulating *T. spiralis* infection given to the Group I mice delayed the action. However, it is important to consider the location of the reductions, as previous work has shown that the defensive mechanisms, stimulated by a prior infection(s) with *T. spiralis*, operate effectively only in the anterior half of the small intestine (Larsh *et al.*, 1952). In the controls (Group III), not given a prior infection with either agent, the majority of the worms were recovered from the anterior half of the small intestine until 9 days after infection. As mentioned earlier, the zenith of the specific inflammatory response is reached at 8

days, and many worms, no doubt, are forced to leave this area. This adds to the abundant evidence on hand that acquired immunity, however feeble, is operating as the result of initial infection with *T. spiralis* (Larsh and Race, 1954; Larsh *et al.*, 1956). In the mice of Group II, given only *A. caninum* prior to the challenging infection with *T. spiralis*, there were more *T. spiralis* worms in the anterior than in the posterior half of the small intestine 3 days after infection. However, in the mice of Group I, given both *A. caninum* and *T. spiralis* prior to the challenging infection with *T. spiralis*, the reverse was true at 3 days after infection. At all necropsy periods, the differences between the 2 groups are not great, but there is a suggestion that the combined effects of the 2 prior infections were greater than the effect of *A. caninum* alone. If additional tests proved this to be the case, it would indicate that both the specific inflammatory factors, known to come into action soon after reinfection, and the non-specific inflammatory factors stimulated by the *A. caninum* infection are involved.

It is clear from the above that a complete picture of worm reduction following a *T. spiralis* infection can not be obtained from counts of worms recovered from the entire small intestine. As pointed out previously, when critical comparisons are to be made it is better to compare counts from the anterior half of the small intestine (Larsh *et al.*, 1952). Many worms recovered from lower levels appear to have been injured by their brief residence in the anterior half and usually remain in the body for only a short time. Others establishing initially in the lower levels of the small intestine probably are not subject to severe actions of the defensive mechanisms, and, as a result, may persist longer. In any event, in measuring the immune state of the mouse host it would seem wise to compare counts from the area of greatest defensive activity.

Larsh and Race (1954) showed that immunity against *T. spiralis* in mice is brought about by a humoral factor(s) followed by secondary cellular cooperation. Coker (1954) showed that when the cellular response in mice was suppressed by cortisone injections, acquired immunity, established before the treatment with cortisone, was eliminated for at least 14 days after a challenging infection. It was concluded, therefore, that the cellular factor(s) is of paramount importance in the actual expulsion of the adult *T. spiralis* worms. The fact that, in the present study, a non-specific cellular response caused a rapid elimination of adult *T. spiralis*, especially from the anterior half of the small intestine, shows that the cellular factor(s) alone is capable of causing the actual elimination of the worms.

SUMMARY

An infection with *Ancylostoma caninum* given 24 to 48 hours prior to an infection with *Trichinella spiralis* caused a significant reduction in the number of adult *T. spiralis* harbored in the entire small intestine of mice, as measured by adult worm counts 7 days after infection. A simultaneous infection with the 2 agents, or an *A. caninum* infection given 12, 96, 144, or 192 hours prior to the *T. spiralis* infection failed to interfere significantly with the establishment and maintenance of *T. spiralis*.

Furthermore, the significant reduction of *T. spiralis* adults occurred by the third day after infection in animals that had received a prior *A. caninum* infection, and by the fifth day in animals receiving both *A. caninum* and *T. spiralis* prior to the challenging infection. By comparing worm counts from each quarter-section of the intestine, it was shown that this reduction occurred primarily in the anterior portions of the small intestine.

Evidence is presented to support the suggestion that the non-specific inflammation, resulting from the *A. caninum* infection, was responsible for interfering with the establishment and maintenance of *T. spiralis*.

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MYRIOTRICHIA SCUTATA BLOMQ. CONSPECIFIC WITH *ECTOCARPUS SUBCORYMBOSUS* HOLDEN *IN* COLLINS

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In the summer of 1953, I discovered a minute, filamentous, brown alga growing epiphytically on the leaves of the "sea grass" *Diplanthera Wrightii* (Aschers.) Aschers. in a muddy inlet near Davis, Carteret County, North Carolina. Because of its striking resemblance to *Myriotrichia occidentalis* Børgesen (1920) of the Virgin Islands, and, more or less, to the other small species of *Myriotrichia*, I concluded that it must belong to this genus, and, furthermore, represented an undescribed species which I named *Myriotrichia scutata* in allusion to its compact, scale-like rhizoidal base. The description of this alga was published in this journal (**70**: 37–41, 1954).

While working on a species of *Ectocarpus*, shortly after the publication of the description of this alga, I ran across an illustration of an alga in Taylor's *Marine Algae of the Northeastern Coast of North America*, Plate 7, figs. 5 and 6, 1937, which appeared to be identical with my new species of *Myriotrichia*. This was *Ectocarpus subcorymbosus*, a manuscript name of Farlow, described by Holden but published by Collins (1905). This has been collected in Massachusetts, Connecticut and Maryland and distributed in both *Algae Exsic. Am. Bor. 197*, and *Phyc. Bor. Amer. 415, 630*. It also grew on a marine angiosperm, *Ruppia maritima*.

In describing two new species of marine algae from Bridgeport, Connecticut, Holden (1899) found one of them (*Stictyosiphon subsimplex*) often associated with *E. subcorymbosus*. After examining a specimen of this alga, my opinion was confirmed that *Myriotrichia scutata* and *Ectocarpus subcorymbosus* are conspecific. My overlooking Taylor's illustration at the time of my discovering this alga on the coast of North Carolina was mainly due to my hasty conclusion that what I had found was not an *Ectocarpus* but something else.

What led me to this conclusion? First of all, the position of the plurilocular sporangia which usually were found to be opposite and even in whorls of three. Furthermore, they were typically borne on the cell which also bears terminally a hyaline filament with a basal growth region. The relation of the origin of this terminal, hyaline filament to branching, including the position of sporangia, was discussed in my paper. The plurilocular sporangia seemed also too few-locular to belong to an *Ectocarpus*, although somewhat more numerous than in most of the smaller species of *Myriotrichia*, except *M. occidentalis*. All of the above characters are more like those of *Myriotrichia* than of *Ectocarpus*. It is, therefore, still my conviction that this alga from the coast of North Carolina belongs to the genus *Myriotrichia* and not to *Ectocarpus*, and, furthermore, it is identical with *Ectocarpus subcorymbosus*. The correct name for this alga should, therefore, be *Myriotrichia subcorymbosa* (Holden *in* Collins) comb. nov.

In discussing in my paper the possible distribution of this alga, I ventured to assume that it had tropical affinities and was probably widely distributed further south than North Carolina. This assumption has been found to be correct, as Dr. H. J. Humm has collected it at several localities on the Gulf Coast of Florida, and has obtained specimens of it from Texas and Vera Cruz, Mexico. In all these localities, it grew epiphytically on various marine angiosperms.

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THE TAXONOMY AND CHROMATOPHORES OF *PYLAIELLA ANTILLARUM* (GRUNOW) DETONI

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I. Introduction.—In 1942, while studying parasitic organisms of marine algae at Beaufort, North Carolina, Karling (1944), collected for the first time in this area a brown, filamentous alga which I determined as *Pylaiella fulvescens* Bornet (1889). Since that time, it has been found to be a permanent inhabitant of this locality, occurring sporadically during the summer months on a variety of substrata in the Beaufort Harbor and as far distant as Cape Lookout (Williams, 1948). Its greatest abundance, however, is found on a peaty shoreline of Harker's Island (Blomquist and Humm, 1946) where it evidently persists during the winter season by its stoloniferous system of filaments.

In examining this alga for the first time, I was attracted by its beautiful and interesting chromatophores and their remarkable variation through the plant. From the available literature, I soon discovered that these chromatophores had been misinterpreted by some authors and that this error had been copied by others. Furthermore, it appeared that the chromatophores were the most distinctive characteristic of this alga. It seemed, therefore, worthwhile to make a study of this alga with a two-fold aim of acquiring a correct understanding of its chromatophores and, if possible, the significance of their variation.

Incidental to this study, the question arose regarding the distribution of this alga, which in turn necessitated a consideration of its taxonomy.

II. Taxonomy.—In 1928, Schousboe collected a brown, filamentous alga on the coast of Morocco, which he named (mss.) *Conferva fulvescens*. In a study of Schousboe's collections of algae, Thuret transferred it to the genus *Ectocarpus* (mss.). In 1870, Bornet and Thuret collected it at Biarritz, France, and observed that it had intercalary sporangia and motile spores. Hoping to acquire a better knowledge of its reproduction and life history, Bornet delayed describing it until 1889 when he published a formal description of it and transferred it to the genus *Pylaiella* under a new subgenus *Bachelotia*. In 1895, Sauvageau reported it also from Guetherey, France, and from San Vincent and Corogne, Spain, and contributed an excellent account of its morphology and reproduction. In 1929, Kuckuck referred to this alga as *Bachelotia fulvescens*, elevating Bornet's subgenus to generic rank, a combination which has not, however, so far become generally accepted.

The first report of this alga from the West Atlantic was that of Collins and Hervey in their *Algae of Bermuda* (1917), and in 1920 Børgesen reported it from the Danish West Indies. Since then, it has been reported from Brazil (Schmidt, 1924), and in 1941 I collected it in Puerto Rico. Recently, Taylor (1954) reports that Dawson has collected it in Cuba.

The first report of the occurrence of this alga on the continental coast of North America was that of Shuh (1932), who identified an alga he collected at Brookline, Maine, as *Pylaiella fulvescens*. I have examined the excellent slides of this collection, deposited in the U. S. National Herbarium, and found that they do not represent this alga but a form of *Pylaiella litoralis* (L.) Kjellman. The first report of this alga from the coast of continental North America is, therefore, that of Karling. Farther south, it has been collected in Florida by McBryde and by Humm.

In the tropical West Atlantic, certain algae have been collected at different times in the past, which evidently represented *Pylaiella fulvescens* but were given other names. In 1852, Harvey described an alga which he named *Ectocarpus Hooperi* for Mr. John Hooper, who had collected it. Unfortunately, Harvey's description as well as his illustration of this alga are so inadequate that it is difficult to determine what alga this represented. However, in 1868, Crouan listed an alga in Maze and Schramm's *Hydrophytes de la Guayane française*, (p. 25) which he assumed was Harvey's species. Bornet examined a small part of this collection and concluded that it represented a *Pylaiella* of the subgenus *Bachelotia*, but, being uncertain as to its further identity, assigned it only to "*Pylaiella* sp." It seems to have been assumed, however, both by DeToni (1895) and by Vickers (1908) that this represented "*Pylaiella Hooperi* (Harvey) Bornet",

a combination for which Bornet was erroneously credited. In 1908, Vickers reported this species from Barbados. Her excellent illustration (Pl. 34) leaves no doubt that her alga was identical with *Pylaiella fulvescens* Bornet. This was also the opinion of Collins and Hervey who said, in reporting *P. fulvescens* from Bermuda, "*P. Hooperi*, Miss Vickers, seems hardly distinct. In comparing the description of *P. fulvescens* by Bornet with that of '*Pylaiella* sp.' (*Ectocarpus Hooperi* Crouan) on the following page, the chief distinctive character of the latter seems to be the *Rhizoclonium*-like ramuli near the base; such ramuli were occasionally seen in the Bermuda plant, but were not at all abundant."

In 1867, Grunow described an alga from Guadeloupe, which he named *Ectocarpus Antillarum*, which also resembles *P. fulvescens* and was transferred to the genus *Pylaiella*, subgenus *Bachelotia*, by DeToni (1895). In a study of the marine algae of Bahamas, Howe (1920) found what seemed to him to be this alga and, therefore, assigned it to Grunow's species (*P. Antillarum* (Grun.) DeToni). In so doing, he said, "The filaments are mostly 25–40μ in diam., while Grunow's description of the type makes the diameter only 11–25μ, but as the base of one of the filaments figured by Grunow is nearly 35μ broad it seems difficult to distinguish the Bahama plant. The later-described *P. fulvescens* (Schousb.) Bornet may be a synonym, but perhaps differs in the more protuberant, sometimes laterally gemminate sporangia."

I have examined the specimens of Howe's plant from the Bahamas, as well as the description and illustrations of *Ectocarpus Antillarum* by Grunow, and have reached the same conclusion as Howe, namely that they represent the same entity, and, furthermore, are indistinguishable from Bornet's and Sauvageau's alga from Europe, Børgesen's from the Danish West Indies, Vickers' from Barbados, and other more recent collections from the West Atlantic, including the one from Beaufort, North Carolina. It is my conclusion, therefore, that all of these different names have been applied to the same entity. Some variation undoubtedly exists, but this seems to be mainly in size and the extent of the stoloniferous system, both of which may be expected to vary in relation to differences in substrata as well as temperature, and the degree of constancy of the medium in which the alga grows.

If the above conclusion is correct, namely that the plants of Crouan, Grunow, Vickers and Howe are the same entity as those of Schousboe and Bornet, the valid name and synonymy of this alga should be as follows:

Pylaiella antillarum (Grunow) DeToni, Syll. Alg. **3**: 535. 1895.

Conferva fulvescens Schousboe, mss. 1828.

Ectocarpus fulvescens Thuret, mss., Algae Schousboeanae, Nos. 109–110.

Ectocarpus (*Pilayella*) *Antillarum* Grunow, Reise Novara Bot. **1**: 46. Pl. 4, f. 2. 1867.

Ectocarpus Hooperi Crouan, *in* Maze and Schramm, Hydrophytes de la Guyane française. p. 25. Not *Ectocarpus Hooperi* Harvey. Ner. Bor.-Amer. **1**: 143. T. 12, f.E. 1852.

Pylaiella fulvescens Bornet, Rev. Gen. Bot. **1**: 8. 1889.

Pylaiella sp. Bornet, *Ibid.* **1**: 9.

"*Pylaiella Hooperi* (Bornet) DeToni." Syll. Alg. **3**: 357. 1895.

Bachelotia fulvescens (Bornet) Kuckuck, Wiss. Meeresuntersuch. Abt. Helgoland. N. F. **17**(2): 21. 1929.

III. MORPHOLOGY.—The general structural features of *Pylaiella antillarum* were first described by Bornet (1889) but more adequately by Sauvageau (1896). However, for the purpose of orientation in discussing its chromatophores it seems best to review briefly its morphology.

This alga is composed of two distinct systems of filaments, the creeping or stoloniferous and the erect (Fig. 1), representing an algal form which has been designated as heterotrichous. The stoloniferous filaments grow exclusively from an apical cell (Fig. 1A, 14, 15) and give rise to three different kinds of branches, viz. (1) those which are similar to the ones from which they come and extend the stoloniferous system over the substratum; (2) short, freely branching filaments of limited growth, the rhizoidal haptera ("crampon" of Sauvageau) which anchor the plant; and (3) the erect filaments which synthesize food and bear the reproductive cells. The erect filaments attain a length of a few centimeters and are more or less twisted together in tufts. They give rise to two kinds of branches, viz. (1) those similar to the ones from which they come and (2) stoloniferous filaments. The latter are formed especially freely on detached portions of the erect filaments (Fig. 1C–1G). They often arise unilaterally so that the parent filament curves, and often two together in adjacent cells ("geminate") (Fig.

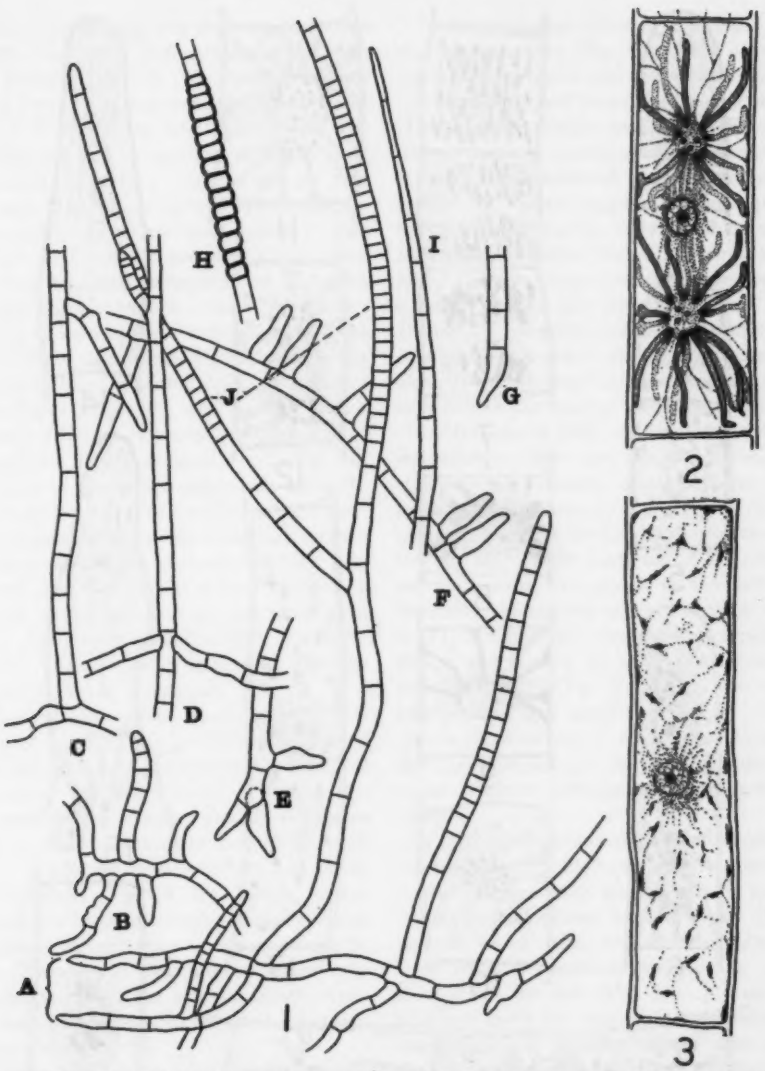

FIG. 1. A–J. Details of habit of *Pylaiella antillarum* (Grun.) Detoni. A, B. Stoloniferous system of filaments showing types of branches. C–G. Portions of erect filaments forming stoloniferous branches. H. Young monolocular sporangia. I. Hyaline terminal portion of an erect filament. J. Intercalary growth regions of erect filaments. ×5. FIG. 2. Mature cell of an erect filament. ×542. FIG. 3. Mature cell of a stoloniferous filament. ×542.

1F). While early growth in the erect filaments is to a limited extent apical (Fig. 4), and to some extent basal, the permanent growth region eventually becomes intercalary (Fig. 1J). It is in the latter growth region that the reproductive cells, the unilocular sporangia, are formed (Fig. 1H). No plurilocular sporangia have ever been observed in this alga. In the Beaufort plants, sporangia have been seen only in immature condition.

IV. THE CHROMATOPHORES.—The most common cell type seen in *Pylaiella antillarum* is

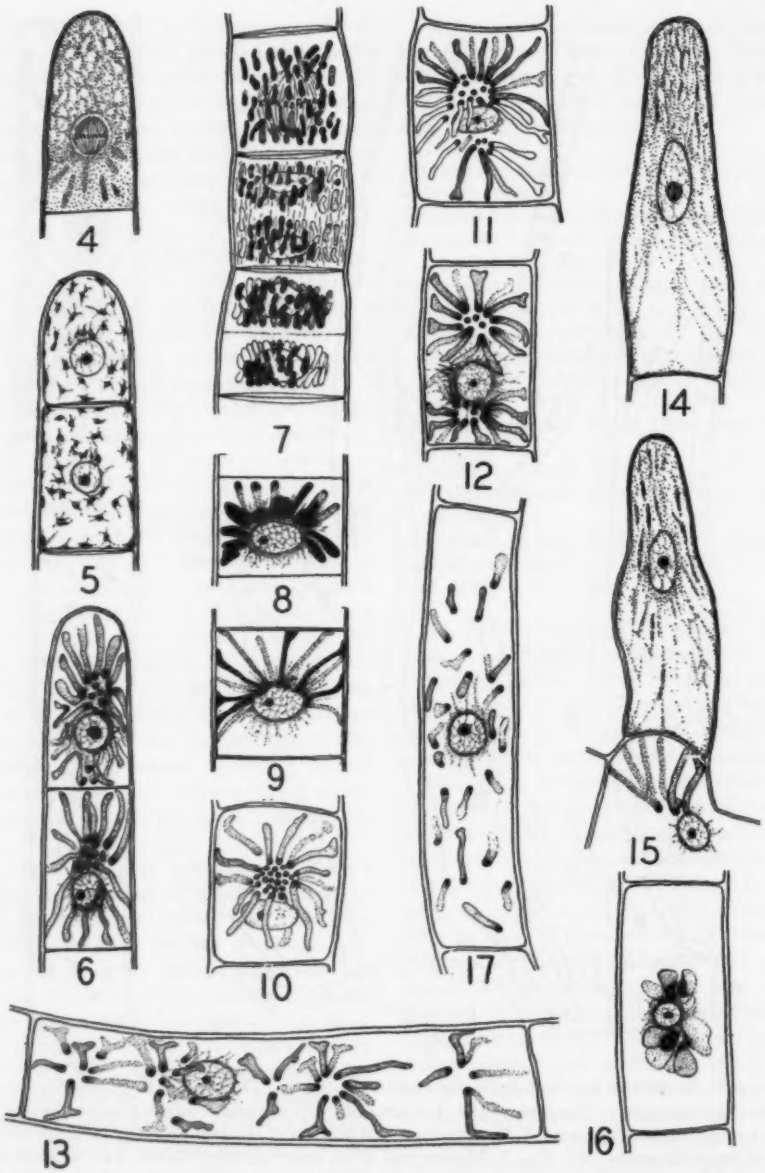

FIG. 4–17. Variation in chromatophores in *Pylaiella antillarum* (Grun.) Detoni. FIG. 4. Terminal embryonic cell of erect filament, which originated from an erect filament. Fig. 5. Two terminal cells of an erect filament, which originated from a stoloniferous filament. Fig. 6. Two terminal cells of an erect filament after cessation of division. 7. Three successive stages in cell division in the intercalary growth region of an erect filament. Fig. 8–12. Successive stages in the formation of new cells in an erect filament. Fig. 13. A cell of an erect filament, which has passed the stage of maturity. Fig. 14. An apical cell of a stoloniferous filament. Fig. 15. A stoloniferous apical cell which has originated from an erect filament. Fig. 16. Degenerate cell of an erect filament. Fig. 17. An old cell of an erect filament. ×542.

characterized by having two star-shaped groups of slender, rod-shaped chromatophores with the nucleus between (Fig. 2). They are bent into *U*-shaped forms with the bend directed toward the center of the group and the ends of the chromatophores are sometimes flattened and slightly lobed where they come in contact with the cell wall. This chromatophore pattern characterizes a mature cell of the erect system of filaments. This was correctly observed by Sauvageau (1896), but was misinterpreted by Børgesen (1920), who thought that the cells had only two star-shaped chromatophores somewhat like those of the freshwater alga *Zygnema*. In immature cells of this system there is at first only one group of chromatophores (Fig. 10), and in cells which have passed the stage of maturity, the groups of chromatophores may increase to 4 or 5 (Fig. 13). In still older or somewhat degenerate cells, the groups of chromatophores usually become completely disorganized so that they lose their characteristic group pattern and are scattered throughout the cell (Fig. 17). In cells of the stoloniferous system, the chromatophores are not rod-shaped but small and evenly distributed in the cytoplasm (Fig. 3) as in other species of *Pylaiella* and certain species of *Ectocarpus*.

1. Embryonic cells.—Although apparently every cell, whether young or old, in the two systems of filaments, is potentially embryonic, the principal growth regions are either apical or intercalary. In the stoloniferous system, growth originates in an apical cell which is quite distinct from the other cells. It is longer, broader at base and tapers somewhat distally (Fig. 14, 15). It has no distinct chromatophores although deeply staining areas in the cytoplasm may represent primordia of these. However, in stoloniferous apical cells which originate from the erect filaments, rod-shaped chromatophores are evident in all stages of disintegration (Fig. 15).

The terminal cell of the erect system is never a distinct apical cell (Fig. 4). As in apical cells of the stoloniferous system, the pattern of its chromatophores indicates where it originated. If formed from a stoloniferous cell, it has small scattered chromatophores like its parent cell (Fig. 5), but if it originated from a cell of the erect system it has short rod-shaped chromatophores. However, these differences soon disappear. After a short period of division, the terminal cell is transformed into a mature cell with two groups of chromatophores (Fig. 6).

In the intercalary growth region, the cells are shorter than wide (Fig. 1J). They have a relatively large nucleus and are densely filled with cytoplasm in which short, rod-shaped chromatophores are distributed throughout the cell without any indication of grouping. When the nucleus divides, these scattered chromatophores divide further into small fragments which apparently become about equally distributed between the two daughter nuclei (Fig. 7). At first, they surround the new nuclei, but they soon become aggregated on one side of each nucleus (Fig. 8). During the completion of the organization of the two new embryonic cells, the chromatophores elongate and resume their scattered pattern characteristic of the "resting" embryonic cell.

2. Maturing of cells.—In the formation of new stoloniferous cells from the apical cell of the stoloniferous filaments, small particles of chromatophores commence appearing in the cytoplasm and become evenly distributed throughout the new cell. In the maturing of the cells in the erect filaments, in contrast to the stoloniferous filaments, the chromatophores undergo considerable transformation. They first aggregate on one side of the nucleus, as in the formation of new embryonic cells (Fig. 8). From this aggregate emerge the long slender *U*-shaped chromatophores some of which commence migrating to the opposite side of the nucleus until about equal numbers compose the two groups (Fig. 9–12).

3. Old and degenerate cells.—In old cells of the erect system of filaments, the chromatophore groups increase from two to four or more with fewer chromatophores in each group (Fig. 13). In still older cells, the chromatophores lose their group organization and become scattered throughout the cell (Fig. 17). In cells of the filaments inside the twisted tufts of filaments the chromatophores become closely aggregated in the two groups and lose their rod-shaped form (Fig. 16). This may be caused by the crowded situation of the filaments, resulting in reduced light intensity as well as availability of nutrients.

SUMMARY

It is concluded that the name *Pylaiella fulvescens* (Schousboe) Bornet, which has generally been applied to a tropical and subtropical, phaeophycean, filamentous alga is a synonym of *P. antillarum* (Grunow) DeToni, as well as some other names which have evidently been applied to the same entity.

The chromatophores in the cells of the erect filaments of this alga are mostly slender rods which in a mature cell are bent into *U*-shaped forms and organized into two groups with the bend of each rod directed toward the center of the group. Cells with only one group of chromatophores are immature, and cells with more than two have passed the stage of maturity. Cells in which the chromatophores are neither *U*-shaped nor grouped, but scattered, are either embryonic or old. Cells in which the groups of chromatophores are so closely aggregated that they have lost their characteristic rod-shaped form appear mostly in the inner filaments of the twisted tufts, and may be considered as more or less degenerate as a result of the crowded situation. The chromatophores in cells of the stoloniferous filaments are not rod-shaped and grouped but small and scattered.

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TAXONOMIC STUDIES ON NORTH AMERICAN MOSSES. VI–XIII[1]

By Howard Crum[2] and Lewis E. Anderson[3]

VI. Ceuthospora gen. nov.

Planta minima, 0.6–2 mm. alta, brevicaulis, pallido-viridis, gregaria, e filamentis confervoideis viridibus et ramosis oriunda. Folia 6–10, conferta in caule brevissimo, infime parva, superne sensim majora, usque ad 1.6 mm. longitudine, sicca plus minus contorta, madida flexuosa, erecto-patentia, apice plus minus recurvato, lanceolato-ligulata, acuta, marginibus erectis, undulatis, supra medium irregulariter et obtuse serrulatis; costa valida, prope basin 24–49 μ latitudine, parce attenuata, subpercurrens, in sectione transversali e strato medio 2–4 cellulis eurycystis et stereidis ventralibus et dorsalibus composita; cellulae basi folii oblongae, pallidae, laeves, ca. 60 × 12 μ, superne sensim minores et viridiores, apice subquadratae vel brevi-oblongae, 8–12(16) × 8 μ, ambabus superficiebus obscure unipapillatae. Dioica. Capsula emersa, cleistocarpa, sphaerica, obtuse apiculata, 297–330 μ lata, 363–396 μ alta, maturitate pallido-brunnea propter colorem sporarum, cellulis oblongo-sexangulatis, parietibus tenuibus, stomatibus superficialibus, paucis, basi extrema capsulae; seta subglobosa, usque ad 190 μ longitudine; calyptra campanulato-mitrata, parce lobata, papillata, ca. 284 μ alta, tertiam vel fere dimidiam partem capsulae obtegens; sporae ovales, 29–32 × 24–29 μ, fusco-brunneae, dense et minute papillatae.

Ceuthospora mexicana (Bartr.) n. comb. *Ephemerum mexicanum* Bartr., The Bryologist **28**: 66. Fig. 14–27. 1925.—Plants pale green, minute, 0.6–2 mm. high, gregarious, rising from a branched persistent protonema. Leaves 6–10, crowded on very short stems, small below, becoming larger toward the stem tip, up to 1.6 mm. long, flexuose and erect-spreading with more or less recurved tips, when dry somewhat contorted, lance-ligulate, acute; margins erect, undulate, irregularly and bluntly serrulate above the middle; costa strong, 24–49 μ wide near base (on same plants, broader in upper leaves), only slightly tapered, subpercurrent, in section showing 2 stereid bands and 2–4 median guide cells; basal cells hyaline, smooth and rectangular, about 60 × 12 μ, gradually becoming smaller and green upward, near apex irregularly subquadrate and short-rectangular, mostly about 8–12(16) × 8 μ, obscurely and bluntly unipapillose on either surface. Dioicous. Capsules cleistocarpous, emersed on a short subglobose seta up to 190 μ long, single or sometimes paired, spherical except for the blunt, conic apiculus, 297–330 × 363–396 μ, when mature light brown (because of spores showing through); exothecial cells thin-walled, oblong-hexagonal; stomata very few at extreme base of urn, superficial; calyptra campanulate-mitrate, only slightly and irregularly lobed, covering ⅓ to nearly ½ the urn (including apiculus), about 284 μ high, laxly areolate and conic-papillose throughout; spores dark brown, ellipsoidal, 29–32 × 24–29 μ, densely and minutely papillose.

Shaded bank of dry wash, east of Mazatlán, Sinaloa, Mexico, *E. B. Bartram 505*, November 29, 1924 (type in herb. Bartram).

This species is, of course, very like an *Ephemerum* in many ways, including its growth from a persistent protonema as well as its size and general appearance and the structure of both gametophyte and sporophyte. The areolation of the leaves and the nature of the low, blunt and obscure papillae on their cells strongly suggest the Pottiaceae, however, and the well-developed costa with two stereid bands separated by a row of guide cells seems clearly indicative of a relationship with the cleistocarpous members of the Trichostomoideae. These include a number of rarely collected and poorly understood species of small genera: *Aschisma*, *Trachycarpidium*, *Uleobryum* and *Tetrapterum*. The present species

[1] For Parts I–V *see* Jour. Elisha Mitchell Sci. Soc. **72**: 276–291, 1956.

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shows a particular resemblance to the New Caledonian *Trachycarpidium*, which also has a persistent protonema. The strongly pustulose exothecium and warty calyptra of *Trachycarpidium*, as well as phytogeographic considerations, argue against combining *Ceuthospora* with it in an inclusive genus.

VII. Leaf variation in *Trichostomum cylindricum* (Bruch) C. M.

Grout (1928–40) said that plate 187 in the *Bryologia Europaea* "represents the narrow leaved form with entire margins found at the base of trees in N. Carolina. On moist cliffs nearby the leaves were so much larger that it challenges belief that they can be the same species. These two forms might well be called f. *angustifolia* and f. *latifolia* [sic]." Since then many collections of this variable species have been made in a wide range of habitats in the Southern Appalachian Mountains, notably in North and South Carolina and in Tennessee and Georgia. So many puzzling variations have been found, some of them appearing so patently distinct, that it seems in order to attempt an evaluation of the North American variants of *T. cylindricum*.

The more normal or so-called typical form (Fig. 2, 3, 6) of *T. cylindricum*, as it occurs over most of its range, (Fig. 8) can be characterized as follows:

> Leaves lanceolate to ligulate, gradually tapering to a slender, pointed apex (somewhat variable in degree of acuteness), spreading and flexuose, when dry crisped and contorted; margins plane, undulate, often sinuose above, crenulate because of marginal papillae, otherwise usually entire but sometimes irregularly notched or dentate, not margined or occasionally with some suggestion of a yellowish border of 1–2 rows of thicker-walled cells; upper cells of blade usually 8–9 μ, strongly papillose and rather obscure, with walls moderately thickened.

Perhaps the most striking variation from the norm is Grout's "f. *latifolia*" (Fig. 4, 5). This is apparently common in the Southern Appalachians, and, judging from the number of collections of it, more abundant there than the typical form. Its distinctive characteristics appear over and over again in the same combinations:

> Leaves ligulate to broadly ligulate, usually abruptly acute, sometimes gradually narrowed to an acute, usually asymmetric apiculus, spreading and scarcely flexuose, when dry somewhat crisped and contorted, but less than in the preceding, brittle and often broken; margins plane, undulate, with 1 or usually 2 rows of thick-walled, less papillose, yellowish cells forming a distinct border, discontinuously crenulate, with the crenulations absent opposite contiguous cell walls, usually irregularly dentate to conspicuously notched near the apex or at the shoulders; upper cells 12–13 μ, thick-walled, with fewer and thicker papillae than in the previous form.

The broad, abruptly apiculate leaves, with well-developed margins (Fig. 7) and larger cells (*cf.* Fig. 6 and 7), strongly suggest var. *daldinianum* (DeNot.) Limpr., which has been recognized in Scotland and northern Italy, and it is not improbable that the Appalachian plants described above could be safely referred to that variety. When well developed, the variation is easily recognized and would deserve at least subspecific rank were it not for the occurrence of more than a few plants with other combinations of characters. For example, some plants match the above in all respects except that they lack the border of yellowish, thick-walled cells. Others lack both the border and the larger cells, thus resembling typical material in every way except for broad leaves. Finally, an undeniable suggestion of a yellowish border can be demonstrated in some plants with relatively narrow leaves and small cells. Thus the characters which appear to differentiate the broad-leaved variation actually occur in all possible combinations with the characters associated with typical *T. cylindricum*.

The extreme variation in the direction of narrow leaves is probably what Grout (*loc. cit*) had in mind as "f. *angustifolia*" (Fig. 1). This form is found not only in the Southern Appalachians but also in other parts of the range and may be characterized as follows:

> Leaves long linear-lanceolate, scarcely ligulate, tapered gradually to long, slender apices, spreading and flexuose, often somewhat secund, when dry strongly curled and spirally contorted as in *Tortella tortuosa;* margins plane, scarcely undulate, not sinuose, minutely and continuously crenulate, entire, with no notches and no suggestion of

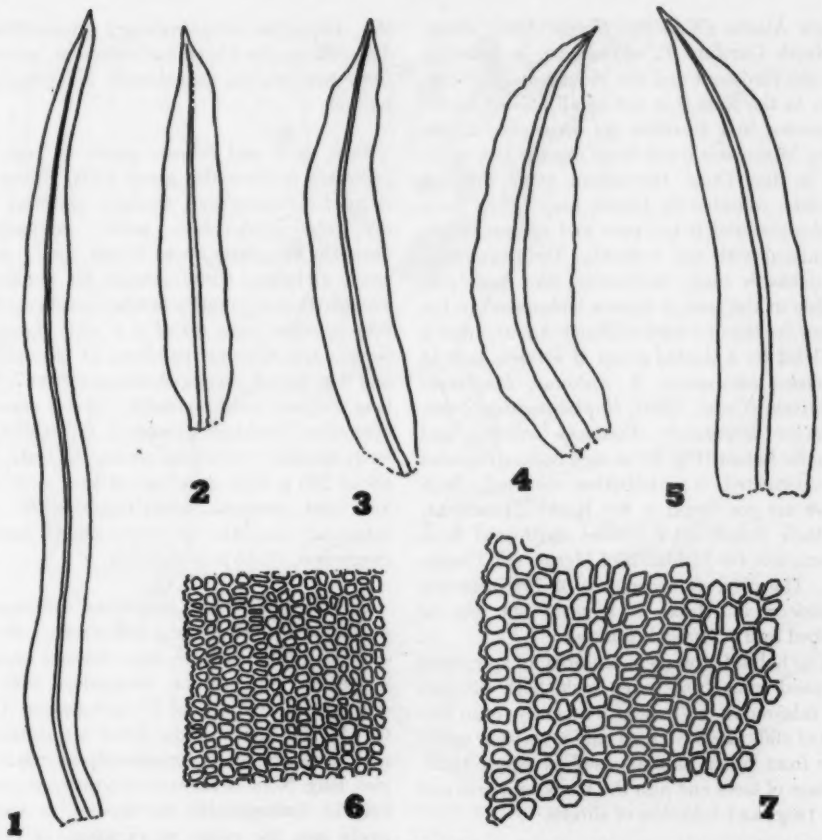

FIG. 1–7. *Trichostomum cylindricum* (Bruch) C. M. Fig. 1. Leaf outline from narrow-leaved form. ×25. Fig. 2–3. Leaf outlines from typical plants. ×25. Fig. 4–5. Leaf outlines from broad-leaved form. ×25. Fig. 6. Median and marginal cells from leaves of typical plants. ×250. Fig. 7. Median and marginal cells from leaves of broad-leaved forms, showing differentiated margin and larger cells. ×250.

dentations, not bordered or rarely with a row of lighter-colored marginal cells; upper laminal cells 8–9 μ, papillose, somewhat obscure.

We have observed all degrees of intermediates between the narrow-leaved form and what we regard as the typical form of *Trichostomum cylindricum*. The broad-leaved forms (Fig. 4, 5) with large leaf cells and a distinct border (Fig. 7) are perhaps easier to distinguish, but the occurrence of intermediates and the lack of complete correlation of distinctive characters make it impractical to recognize an additional entity segregated from the variability of the species.

According to Grout (*loc. cit.*) *T. cylindricum* is found "on moist rocks and even in brooks in cool shaded places in hills and mountains; also on bases of stumps and trees; Greenland to Manitoba and south to the mountains of North Carolina and Arizona." This statement requires amendment. We have seen specimens from Arizona, Mexico (Distrito Federal, Puebla, Chiapas), Guatemala and Ecuador, Alaska, Georgia, Indiana, Iowa, Maryland, Michigan, Missouri, North Carolina, New Hampshire, New York, Ontario, Pennsylvania, Quebec, South Carolina, Tennessee, Vermont, Virginia and West Virginia, but none from Greenland or Manitoba. The range (Fig. 8) as we know it, therefore, is distinctly eastern temperate, extending from Quebec and Ontario southward to Rabun Bald in Georgia and the Ozark Plateau of Missouri, with disjunctions in Arizona and southward and in south-

eastern Alaska (Whittier, *Harvill 565a*, MICH). In North Carolina *T. cylindricum* is found in both the Piedmont and the Mountains, and elsewhere in the East it is not at all limited to the mountains, and therefore its occurrence in the Rocky Mountains is not to be expected. A specimen in the Duke Herbarium from Tolland, Colorado, collected by Grout, may belong here, but the material is too poor and scrappy to be determined with any certainty. The disjunction in Arizona is easily understood and quite predictable in the case of species widespread in the eastern deciduous forests of North America and is paralleled by a sizable group of species, such as *Anomodon attenuatus*, *A. rostratus*, *Lindbergia brachyptera* (Crum, 1956), *Haplohymenium triste*, *Thuidium delicatulum*, *Fissidens cristatus* and *Hyophila tortula* (Fig. 9), as well as many species more restricted in distribution eastward. Such species are not found in the Rocky Mountains, but their ranges often extend southward from Arizona into the highlands of Mexico and Guatemala. The disjunct occurrence of *Trichostomum cylindricum* in Alaska is unusual but can be matched by *Thuidium delicatulum*.

As to habitat, the great majority of specimens examined came from moist rock, both siliceous and calcareous, and very frequently from the faces of cliffs; we have seen only a very few specimens from other habitats, soil, a peaty bank, the base of trees and also the trunk of a tree and even twigs and branches of shrubs.

VIII. Notes on *Trichostomum spirale* Grout

Previously known only from a single locality in Wisconsin (and probably from a single collection there), this species was recently recognized in one of John Macoun's collections at the National Museum of Canada rather inadequately labeled "Flora Canadensis, No. 25. Hastings Co." and named "*Weissia viridula* var." Two specimens are to be found in Grout's herbarium, both marked type (in Grout's writing) and labeled "on rotten wood, St. Croix Falls, Polk Co., *C. F. Baker*;" one labeled in Grout's hand is dated March ? 1897, and the other rather illegibly in some other hand bears the date November 1897. The fact that both show the sporophytes in the same stage of development (most capsules lacking opercula but still retaining spores) makes it seem doubtful that two collections separated by several months were actually made.

Grout's very brief description of *T. spirale* (in his *Moss Flora*, vol. 1, p. 162) lacks many important details, particularly of measurement; the following supplementary description is drawn from the Canadian collection, as well as from the original specimen(s) in Grout's herbarium:

Very small and delicate plants in loose, dull, yellowish or brownish green tufts. Stems very short. Leaves concave, strongly contorted when dry, oblong-lanceolate, acute, gradually or abruptly apiculate, up to 2 mm. long; margins erect, undulate, entire (except for papillae) or irregularly and minutely notched; costa excurrent into apiculus; cells about 8 μ wide, hexagonal, densely and minutely papillose, at base pale, lax and thin-walled, oblong. Autoicous. Seta 7–8 mm. long. Capsule erect, cylindric, 1.2–1.3 mm. long; operculum slenderly rostrate, 0.7–0.8(1) mm. long; annulus persistent; peristome teeth erect, about 250 μ high, papillose at tips, with rather low, faint, irregular, spiral ridges below; spores spherical, smooth or very finely papillose-roughened, 11–15 μ in diameter.

In addition to the important differences in peristome sculpturing and inflorescence the very short stems and smaller, more delicate leaves are not easily confused on comparison with even depauperate material of *T. cylindricum* (Bruch) C. M. The leaves of the latter are usually 3–4 mm. long and only occasionally as much as 6 mm. long. None of the sporophytic measurements help in distinguishing the species, as they fall easily into the range of variation of *T. cylindricum:* seta 9–11 mm., urn 1.25–1.6 mm., operculum 1 mm., peristome teeth 160–270 μ, spores 12–15, occasionally 18 μ.

IX. The American distribution of *Hyophila tortula* (Schwaegr.) Hampe

Sharp (1955) recently reported finding *Hyophila tortula* in northern Michigan where it grew on boulders along Au Train Bay of Lake Superior. Although rather local in distribution, the species is much more widely distributed in the Americas than Sharp's map indicates, and we have summarized the range in Fig. 9, which is based on specimens from all the leading American herbaria studied over a period of ten years. (Records from northern South America are not included.) A number of range extensions and a few other records not well publicized previously are listed below:

ONTARIO: On limestone rocks along the Syden, ham River, Owen Sound, *J. Macoun*, Sept. 17-

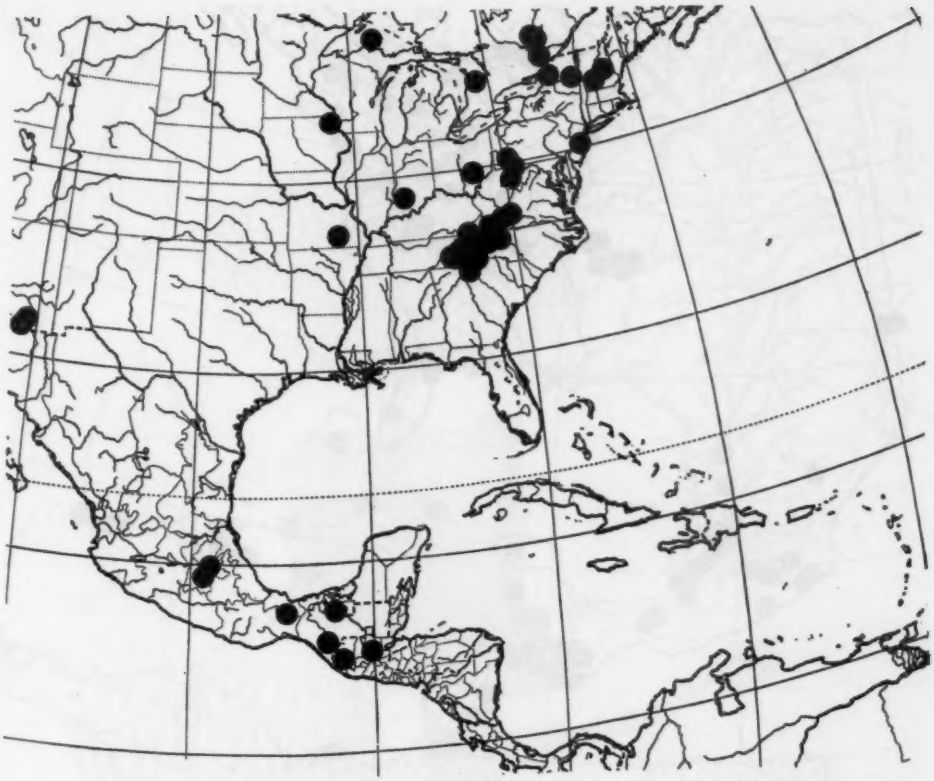

FIG. 8. Distribution of *Trichostomum cylindricum* (Bruch) C. M.

1890, as *Didymodon macounii* Kindb. and *Leptodontium canadense* Kindb. (CAN). VERMONT: On wet limestone rocks below old dam, Norwich, Windsor Co., *Ethel Hutchinson*, Nov. 2, 1949 (DUKE). OHIO: On soil near stream, North Appalachian Experimental Watershed, near Coshocton, Coshocton Co., *H. N. Moldenke 14433* (DUKE). Kelly's Island, Erie Co., *Frances Wynne*, June 9, 10, 1945 (CAN). INDIANA: On limestone, along Nine Penny Creek, Tunnel Mill Reservation, Clark Co., *W. D. Gray 1017* (WELCH). IOWA: 12 mi. from Dubuque, Dubuque Co., *H. S. Conard*, Apr. 18, 1937 (IA). On blocks of stone at base of ledges, Wapsipinicon State Park, Jones Co., *B. Shimek*, June 13, 1928 (IA). WEST VIRGINIA: On moist rocks by stream, 941 ft. alt., near Uffington, Monongalia Co., *Sarah Bailey*, Aug. 3, 1939 (DUKE). VIRGINIA: On moist rocks in stream, 1000 ft. alt., Pinnacles of Dan, W. of Stuart, Patrick Co., *L. E. Anderson & P. M. Patterson 11285* (DUKE). TENNESSEE: On limestone rock in narrow ravine, near Paradise Hill Rd., 2 mi. SE. of Clarksville, Montgomery Co., *A. Clebsch 920* (DUKE). MISSOURI: On moist dolomite, Pulltight Springs, N. of Eminence, Shannon Co., *L. E. Anderson 12510, 12534* (DUKE). On moist limestone, edge of spring, Big Springs, near Van Buren, Carter Co., *L. E. Anderson 12426* (DUKE). ARKANSAS: On moist dolomite, mouth of cave, Blanchard Springs, N. of Allison, Stone Co., *L. E. Anderson 11671, 11708* (DUKE). On dripping ledge in cedar barren, along White River, Sylamore, Johnson Co., *L. E. Anderson 11809* (DUKE). On moist limestone, edge of stream, 12 mi. E. of Springdale, Washington Co., *L. E. Anderson 12292* (DUKE). On limestone, shaded cove, Lost Valley, E. of Boxley, Newton Co., *L. E. Anderson 12179b* (DUKE). FLORIDA: Wahoo, Citrus Co., *Ruth Schornherst 1969* (ABSM).

X. Tortula cainii n. sp.

Planta synoica, dense caespitosa, 1–2 cm. alta, fusco-viridis, infime radiculosa; radiculae copiosae, laeves, brunneo-rubidae. Caulis sine fasciculo medio. Folia sicca superne obscurata, brunneo-virentia, infime nitide brunnea, erecta, apicibus carinatis vel plicatis et incurvatis,

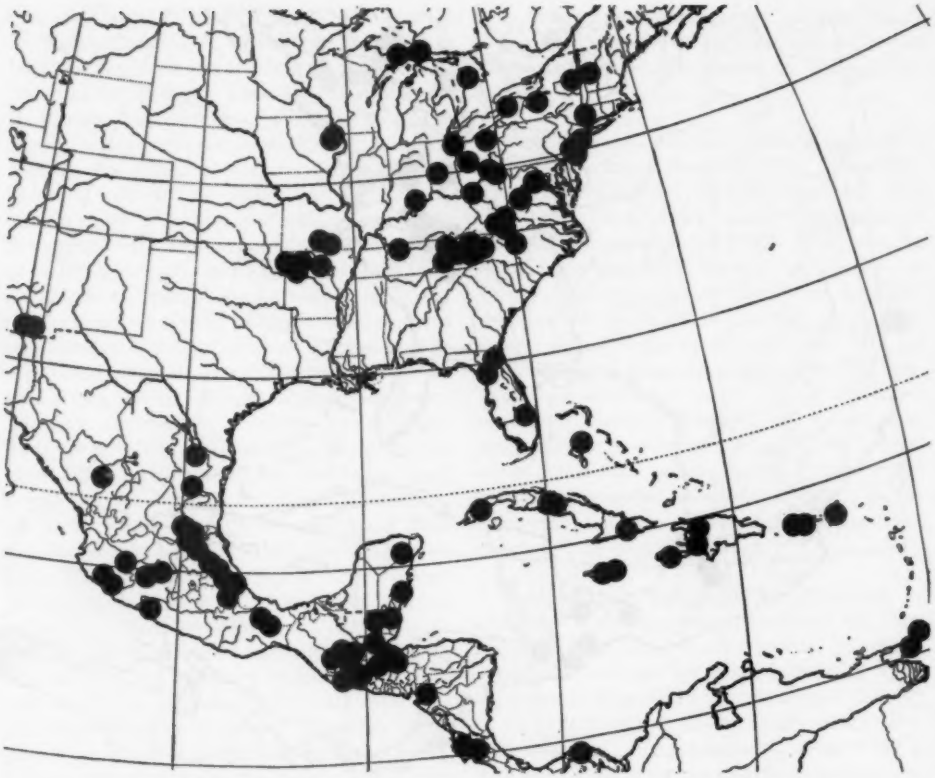

FIG. 9. Distribution of *Hyophila tortula* (Schwaegr.) Hampe

madida patentia, apicibus subsquarrosis, oblonga vel oblongo-ovata, 1.5–2 mm. longa, 0.8–0.9 mm. lata, obtusa, in apiculo carinato et recurvato terminata, marginibus integris, anguste revolutis usque ad medium folii vel ad duas partes e tribus longitudinis; costa in apiculo extensa vel 7–8 cellulis infra apicem evanida, dorso papillosa, basi ca. 70 μ lata, haud attenuata; cellulae hexagoneae vel subquadratae, parietibus in angulis plus minus incrassatis, 3–5 papillis in superficiebus cellularum singularum; papillae demissae inconspicuae; cellulae basilares majores, oblongae, brunneo-luteae, laeves. Caetera ignota.

Plants densely tufted, 1–2 cm. high, brownish-green, with numerous reddish-brown, smooth radicles below. Stems without central strand. Leaves when dry dull and greenish-brown above but shiny brown at the base, erect with tips incurved and strongly keeled or folded, when moist spreading with subsquarrose tips, oblong to oblong-ovate, 1.5–2 mm. long, 0.8–0.9 mm. wide, obtuse and ending in a stout mucro which is pinched and more or less recurved; margins entire, narrowly revolute in the lower ½ or ⅔; costa ending as much as 7–8 cells below the apex but usually disappearing in the folded mucro and thus appearing percurrent, papillose at back, about 70 μ wide at the base and scarcely tapered above, in section showing a well-developed dorsal stereid band, 4 median guide cells and a small group of moderately enlarged, empty ventral cells; upper cells of leaf hexagonal and subquadrate, with brown, rather thin but firm walls often slightly thickened at the corners (the corner thickenings are more conspicuous near the area of differentiated basal cells), 12–18 μ in diameter, mostly isodiametric but often slightly longer than wide (with an occasional cell as long as 24 μ), papillae low and inconspicuous, *C*-shaped, 3–5 per cell, the inner basal cells forming well-differentiated, brownish-yellow groups, oblong

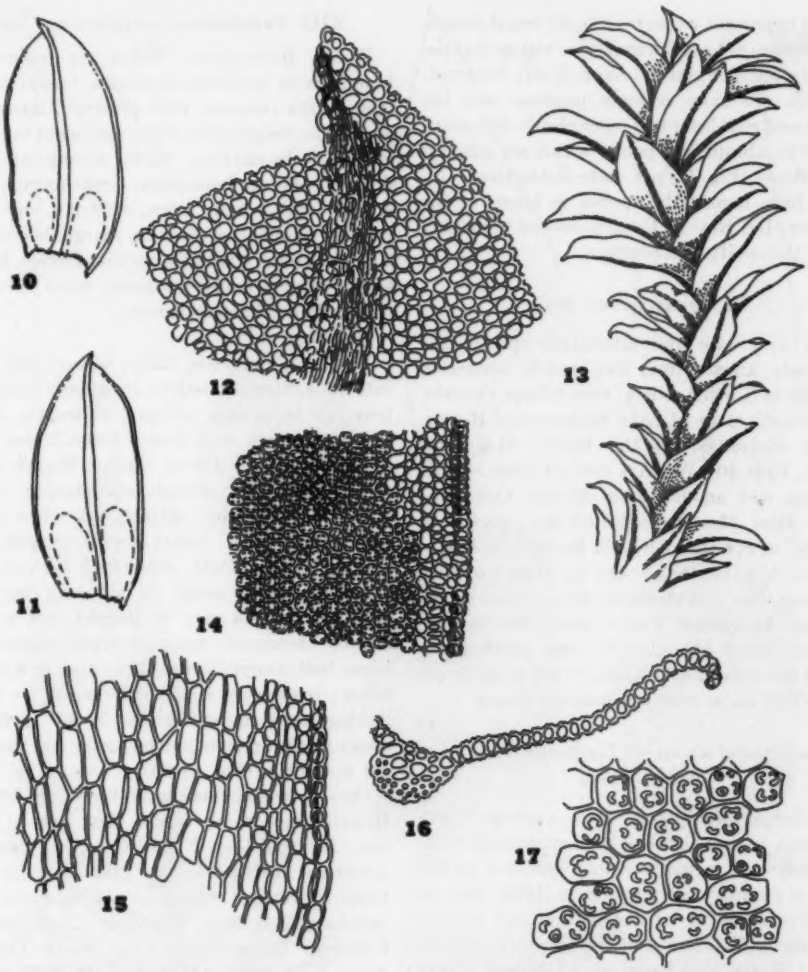

FIG. 10–17. *Tortula cainii* Crum and Anderson. Fig. 10–11. Stem leaves. ×20. Fig. 12. Leaf apex, showing the characteristic abrupt leaf point which is pinched and reflexed. ×300. Fig. 13. Habit sketch of portion of stem. ×35. Fig. 14. Portion of leaf from upper one-third of leaf, showing margin. ×250. Fig. 15. Same, near base. ×250. Fig. 16. Portion of leaf cross-section, showing costa and portion of lamina. ×250. Fig. 17. Enlarged drawing of leaf cells, showing shape and arrangement of papillae. ×525.

ellipsoidal to rectangular, 16–18 × 37–38 μ. Synoicous; paraphyses filiform. Sporophyte unknown. (Fig. 10–17).

On flat limestone rock, Buck Lake, near Sparrow Lake, Simcoe Co., Ontario, Canada, *R. F. Cain 1035*, May 29, 1941 (type *in herb.* Duke University).

We are indebted to Mr. James Kucyniak for drawing our attention to this species which had been distributed by the University of Toronto Cryptogamic Herbarium as *Encalypta vulgaris* var. *mutica* Brid. The plants resemble *Encalypta* in some respects, but the lax, pale and enlarged cells in well-defined basal groups, as well as the costal section and the nature of the papillae, leave little question as to their true position in *Tortula*. Among North American species of *Tortula*, this species bears the greatest resemblance to *T. latifolia* Bruch but differs in

several important respects: it lacks brood-bodies; its leaves are not at all spatulate or rounded at the tips; the leaf margins are more clearly recurved; the cells are more strongly papillose and the inner basal cells laxer and more clearly differentiated. The abrupt leaf points which are pinched and reflexed (Fig. 12) are quite distinctive.

We have named the species in honor of the collector, Dr. Roy F. Cain, professor of botany at the University of Toronto.

XI. *Bryum blindii* BSG

This very rare and interesting species was previously known from two widely separated localities in North America: Port Arthur, Ontario, on the north shore of Lake Superior and Hector, British Columbia, in the Rocky Mountains (Grout, 1928–40). We have verified those records and can add another from British Columbia, where John Macoun collected the species on "debris" at Six Mile Creek in the Selkirk Mountains on Aug. 14, 1885 (CAN). In August of 1956 we made two collections of the species in the Nipigon Provincial Forest near Macdiarmid, Ontario, about 102 miles by road northeast of Port Arthur; both collections (*Crum & Anderson 7819, 7842*) came from wet roadside gravel.

XII. Additional records of *Lindbergia brachyptera* (Mitt.) Kindb.

Lindbergia brachyptera is a curious North American moss which has been considered quite rare, but it is actually rather common in the East, as recently shown by Crum (1956) after an extensive study of its distribution and habitat preferences. This is one of the species of eastern United States also found as a disjunct in the Arizona highlands and represented by very close relatives in the more ancient parts of Eurasia; its range is suggestive of Arctotertiary origins, and its exact distribution is, therefore, a matter of some phytogeographic interest. The following range extensions seem worthy of brief report: Chippewa Co., Michigan (*Crum*, June 2, 1955); New Liskeard, Ontario (*Crum 6344*); Jefferson Co., New York (*Crum 7988, 7990*); Stratford Co. (*Crum & Anderson 7971*), Coos Co. (*Crum & Anderson 7915, 7970*) and Grafton Co. (*Crum 7983*), New Hampshire; Cherokee Co., North Carolina (*Anderson 8107*). The species was not known previously from New Hampshire or North Carolina.

XIII. **Taxiphyllum howellianum** n. sp.

Planta flavo-virens, nitida, in caespitibus humilibus et intricatis deplanata. Caulis repens, irregulariter ramosus; rami prostrati, inaequales, ad 18 mm. longitudine. Folia caulina et ramulina similia, subsquarrosa, valde complanata, undulata, oblongo-lanceolata, cultriformia, late acuta, 1.1–1.5 mm. longa, 0.43–0.5 mm. lata, non vel minimo decurrentia, marginibus erectis, supra medium serrulatis, nervis duobus, brevissimis; cellulae lineari-flexuosae, apice breviores, rhomboideae. Caetera ignota.

Plants yellow-green, shiny, in low, flat, intricate mats. Stems prostrate, irregularly branched; branches horizontal, unequal in length, mostly 10–18 mm. long, with leaves about 3 mm. wide. Stem and branch leaves similar. Branch leaves nearly squarrose, strongly complanate, clearly undulate when dry, slightly so when moist, oblong-lanceolate, lateral leaves curved-asymmetric, broadly acute, about 0.43–0.5 mm. wide near base, mostly about 1.4 mm. long (but ranging from 1.1–1.5 mm. in length), not or very slightly decurrent; margins erect, serrulate in upper half, sharply so near tip, entire or sinuolate below; costa very short and double, or nearly lacking; cells linear-flexuose, 50–80 × 5 μ in upper median region, much shorter and rhomboidal at leaf tip. (Fig. 18–26).

On soil, hemlock-hardwood forest, alt. 3800 ft., Howell Tract, below Cowee Gap, west of Cashiers, Jackson Co., North Carolina, *Lewis E. Anderson 11071*, July 28, 1952 (type in herb. Duke University). On moist shaded rocks, hemlock-hardwood cove, Toxaway Gorge, SE. of Rosman, Transylvania Co., North Carolina, *Lewis E. Anderson 12897*, Aug. 26, 1957.

This species can be recognized at a glance because of its strongly asymmetric, complanate leaves which are glossy and transversely undulate. An attractive species, it has much the appearance of *Plagiothecium neckeroideum* BSG, but the leaf and stem structure are quite different. In the latter, the leaves are clearly ovate and, though also asymmetric, not at all cultriform, and the margins are only very finely serrulate near the narrower tips. Most important in regard to relationships, the leaves of *P. neckeroideum* are strongly decurrent, with basal and alar cells notably enlarged, and the outer stem cells are also broad and lax. In *Taxiphyllum howellianum*

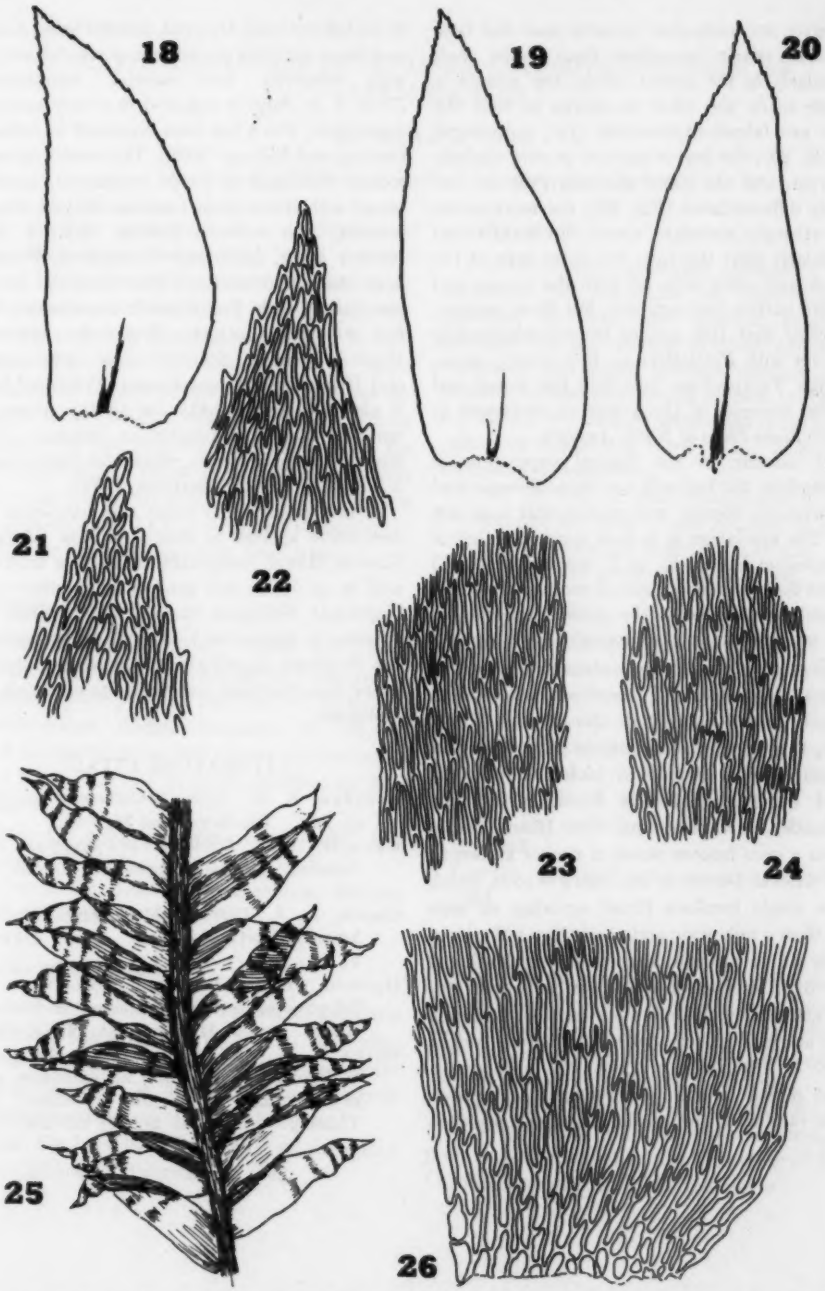

FIG. 18–26. *Plagiothecium howellianum* Crum and Anderson. Fig. 18–19. Stem leaves. ×50. Fig. 20. Branch leaf. ×50. Fig. 21–22. Leaf apices, showing conspicuous marginal teeth. ×300. Fig. 23. Median leaf cells. ×300. Fig. 24. Median basal cells. ×300. Fig. 25. Habit sketch of portion of stem. ×20. Fig. 26. Portion of leaf base, showing insertion. ×300.

the leaves are somewhat broader near the base but more nearly lanceolate than ovate, and, particularly in the lateral leaves, one margin is concave while the other is convex so that the leaves are falcate-asymmetric (*i.e.*, cultriform) (Fig. 21, 25); the leaves are not or very slightly decurrent, and the basal and alar cells are not notably differentiated (Fig. 26); the margins are quite strongly serrulate above the middle and particularly near the tips; the outer cells of the stem do not often strip off with the leaves, and they are narrow and compact. For these reasons, it is clear that this species has no relationship whatever with *Plagiothecium*, in a narrow sense, although *Taxiphyllum* falls into the broad and artificial concept of the genus as employed in Grout's *Moss Flora of North America*.

Very similar to the typical expression in *Taxiphyllum*, the leaf cells are linear-flexuose and conspicuously shorter and rhomboidal near the apex. The areolation is, in fact, much like that of *T. geophilum* (Aust.) Fl. or *T. deplanatum* (Sull.) Fl., but there is little chance of confusion because the undulate leaves of the present species are more asymmetric and less crowded, and also the margins are somewhat more strongly toothed.

The type locality of *T. howellianum* is a virgin hemlock forest known as the Howell Tract, which, through the generosity of Mrs. Margaret Cannon Howell, is now a biological preserve owned by the Highlands Biological Station, Highlands, North Carolina. The Howell Tract adjoins a once famous stand of timber known as the Primeval Forest or Ravenel's Woods, which was a virgin hemlock forest covering an area more than a mile wide and nearly two miles long. Nearly all of this splendid forest was lumbered more than twelve years ago and is now in the early stages of secondary succession. The Howell Tract and a small segment owned by the Wright family of Highlands are now the only undisturbed remnants of this once magnificent forest.

The rainfall of the region averages more than 80 inches per year, the peak months being August and January. This abundance of rainfall coupled with relatively low summer temperatures (70.4° F. in July) is reflected in a very luxuriant vegetation, which has been described in detail by Oosting and Billings (1939). The most important woody dominant is *Tsuga canadensis*, which is mixed with *Betula lenta*, *Castanea dentata*, *Quercus borealis*, *Acer rubrum*, *Halesia carolina*, *Amelanchier laevis*, *Liriodendron tulipifera*, *Magnolia acuminata*, *M. fraseri* and *Ilex monticola*. In close association with *Taxiphyllum howellianum* were the following mosses: *Brotherella recurvans*, *Hypnum fertile*, *Sematophyllum carolinianum* and *Heterophyllium nemorosum*. The same forest is also the type locality for another moss, apparently also on Appalachian endemic, *Schlotheimia lancifolia* Bartr., which was discovered by A. J. Sharp in 1931 (Bartram, 1932).

It is a pleasure to name this handsome and distinctive species in honor of Mrs. Margaret Cannon Howell, naturalist at heart, a trustee as well as an active and generous supporter of the Highlands Biological Station, an unselfish contributor to numerous biological field projects in the Southern Appalachian Mountains, devoted friend and charming companion to countless field biologists.

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LATIN DIAGNOSES OF EARLIER DESCRIBED SPECIES OF TREMELLALES

By Lindsay S. Olive

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In my first paper, containing descriptions of jelly fungi from Chapel Hill, North Carolina (Jour. Elisha Mitchell Sci. Soc. **60**: 17–26, 1944), I reported a number of new species, but neglected to give their descriptions in Latin. In compliance with the International Code, these descriptions are hereby presented. A species described in the original article as *Gloeotulasnella cystidiocarpa* was recently redescribed under the more appropriate name of *Tulasnella conidiata* Olive (Mycologia **49**: 675, 1957) and is therefore not included here. I am grateful to Dr. Donald P. Rogers for preparing the Latin translations.

Platygloea **unispora**

Fructificationes ceraceae, luteae vel brunnescentes, applanato-pulvinatae, ad 1 mm diam. Hyphae nodosae; basidia continua vel septis 1–3 evanescentibus divisa, unispora, 6.1–7.6 × 61–83 μ; basidiosporae late ovatae vel obovatae, 10–13.7 × 13.7–18.3 μ, post abjectionem 2–4-cellulares.

Type: On cedar branches, Chapel Hill, December 11, 1943;

Cotype: On dead cedar branches, Athens, Georgia, January 18, 1946.

Tremella **grandibasidia**

Firme gelatinosa, repanda vel erecta, nodularis vel lobata, lutea, ad 3 cm lata, 6 mm alta. Hyphae nodosae; basidia ovalia, septis frequenter obliquis, 21.4–30.5 × 30.5–42.7 μ; basidiosporae subglobosae vel globosae, 15.3–16.8 μ diam.

Type: On bark of fallen branch of deciduous tree (Mimosa?) December 11, 1943.

Exidia **cokeri**

Fructificationes pustulatae, pustulis in massas nodulatas vel undulatas vel convolutas, applanatas, confluentibus, molliter gelatinosae, fumosae vel ferrugineae. Hyphae nodosae vel enodosae, paraphyses ramosae, demum granulis brunneis incrustatae; basidia globosa vel oblonga, 8.7–12.2 × 9.2–15.3 μ; basidiosporae cylindricae, rectae vel reniformes, 3.8–7 × 8.7–15.3 μ; nonnumquam 1–4-septatae.

Type: On bark of dead limb, probably beech, December 27, 1943 (Form A). The two other collections obtained in the same locality on the same date are designated as cotypes.

Sebacina **sordida**

Effusa, ceracea-gelatinosa vel molliter gelatinosa, superficie irregulari, 200–800 μ crass., fumoso-alutacea vel dilute brunnes-olivacea. Hyphae nodosae; paraphyses fugaces; basidia seriatim e hyphis rectis producta, globosa vel pyriformia, 9–9.5 × 9.2–12.2 μ; basidiosporae reniformes, 4.1–4.6 × 10.4–11.6 μ.

Type: On decorticate pine limb, December 27, 1943;

Cotype: On dead sycamore limb, March 13, 1944.

Sebacina epigaea var. **bicolor**

Firme ceraceo-gelatinosa, hymenium siccum, maculas ferrugineas efformans super subiculum dilute luteum. Basidia 16.8–19 × 21.4–26.9 μ; basidiosporae 9.1–11.4 × 13.7–20 μ, nonnullae in cellulas irregulares resides transformatae.

Type: On rotting oak log, December 21, 1943.

Type specimens of the new species are on deposit in the herbarium of the University of North Carolina and at the Farlow Herbarium.

SOME LIGNICOLOUS MARINE FUNGI FROM THE NORTH CAROLINA COAST

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The investigations by Cribb and Cribb (1956), Johnson (1956a), Meyers (1957), and Miss Wilson (1956) describe several new taxa of halophilic fungi. The number of these new species testifies to the diversity of marine fungi; the discovery, in widely separated coastal regions, of numerous previously described species is indicative of their ubiquity. In the Beaufort Inlet region, North Carolina, for example, seventeen species previously known from more northerly or southerly waters have been found. This paper describes some additional species recovered from submerged wood since the first report of lignicolous fungi from the Beaufort region was made (Johnson, 1956a).

Piricauda pelagica sp. nov. Fig. 10–18

Hyphis atro-brunneis, cum muris crassis, superficialibus ac intracellularibus. Conidiophoris brevibus vel nullis, simplicis, lateralibus, atro-brunneis. Conidiis atris, singularibus, acrogenis, sessilibus, inaequalateralo-obpyriformibus, raro ovoideis vel sphaericis, muriformibus, 34–44 × 17–31 μ, plerumque 36–41 × 19–22 μ.

Vegetative hyphae dark brown, thick walled, superficial and intracellular; sparingly branched. Conidiophores short or lacking, when present, 1–3-celled, lateral, simple, concolorous with the hyphae. Conidia sparingly produced, dark brown becoming black except for basal cells; asymmetrically obpyriform, rarely ovoid or spherical, very rarely cylindrical; muriform; originating apically on short conidiophores, or directly from vegetative hyphae either laterally or terminally; 34–44 × 17–31 μ, predominantly 36–41 × 19–22 μ; germinating by one or more slender, yellow-brown germ tubes.

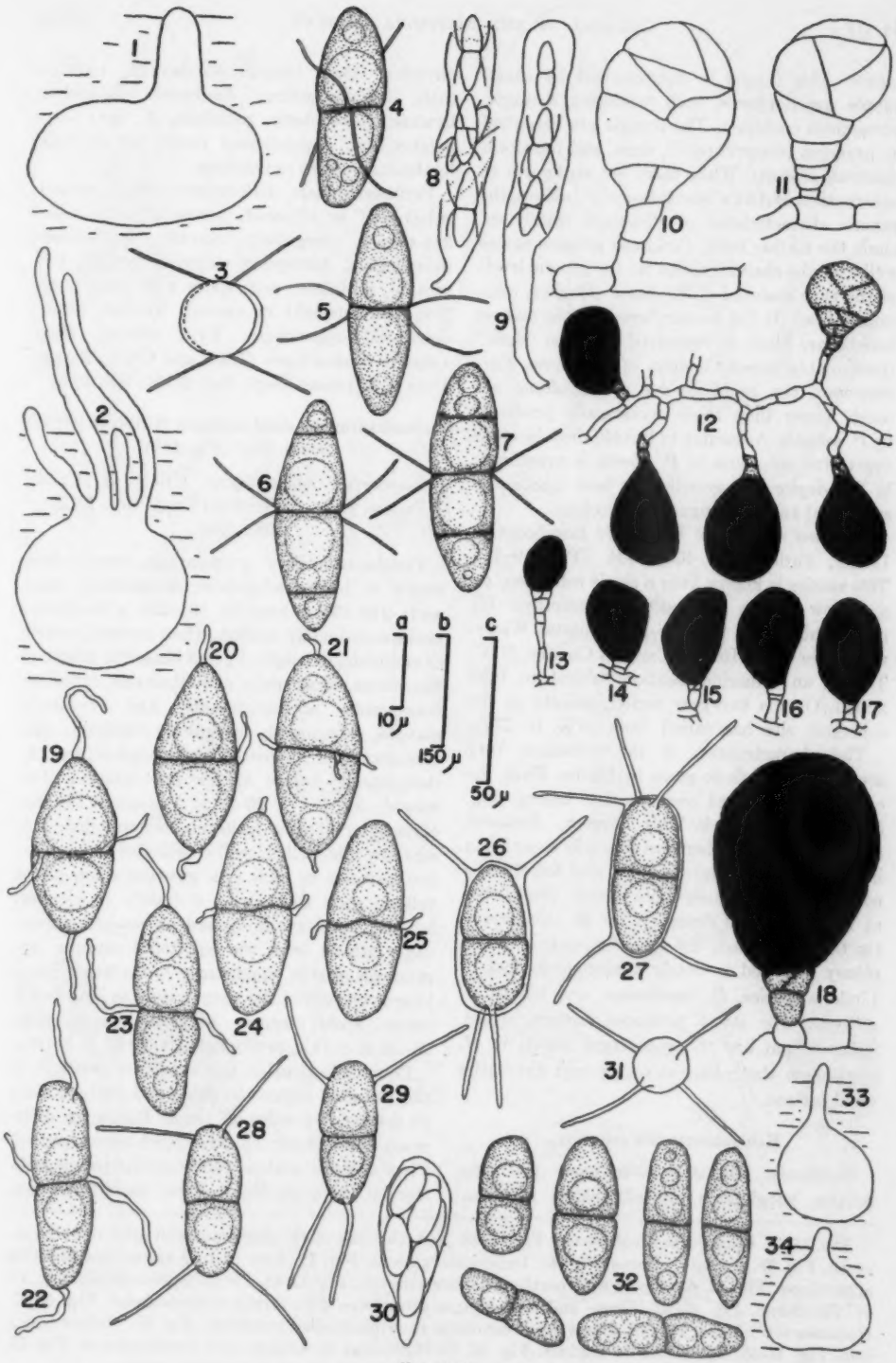
On sunken driftwood, from 18 meters, 1½ miles off Fort Macon Point, Bogue Banks, N. C., July 28, 1956 (TYPE); on pine panels, Annapolis, Maryland, and Fort Amador, Panama Canal Zone, *leg.* S. P. Meyers.

The mycelium is sparse superficially, but profuse deep within the substratum. The conidial characteristics are distinctive. Conidia are always single, usually sessile, and generally asymmetrically obpyriform (Fig. 12) although variations in shape do occur (Fig. 13–17). Immature conidia are yellow-brown; during maturation they darken, and, at maturity, are coal black except for the basal cells (Fig. 18). Only the younger conidia show the muriform septation pattern (Fig. 10, 11). The septa are few; occasionally only one longitudinal septum (in addition to 2–4 transverse ones) is present. The conidial wall is constricted at the septa.

Pure cultures of *Piricauda pelagica* were not obtained. Neither conidia nor hyphal fragments germinated on seawater agar, or on media fortified with carbon and nitrogen sources. The addition of biotin, riboflavin, and thiamin to the media, similarly, did not induce germination. Cold treatments prior to seeding the conidia also failed to stimulate germination. Conidia germinated sparingly in sea water, however, provided they were completely submerged, but development of the germ tubes did not proceed beyond primary branching.

The affinities of *Piricauda pelagica* are with *Stigmella*. Bubák (1914), however, recognized that one species of *Stigmella*, *S. uleana* Saccardo and Sydow, did not correspond with the accepted generic limits. Consequently, he proposed the new genus *Piricauda*, with a single species, *P.*

Fig. 1–9. *Halosphaeriopsis mediosetigera*. Fig. 1, 2. Perithecia. Fig. 3. Terminal view of ascospore. Fig. 4–7. Ascospores showing variations in appendages and septation pattern. Fig. 8, 9. Asci. Fig. 10–18. *Piricauda pelagica*. Fig. 10, 11. Muriform conidia; immature. Fig. 12. Configuration of conidia and conidiophores. Fig. 13–17. Variations in shape of mature conidia. Fig. 18. Mature conidium showing black apical portion and lighter basal cells. Fig. 19–25. *Halosphaeria appendiculata*. Fig. 19–25. Variations in gelatinous terminal and meridional appendages, and in shape of ascospores. Fig. 26–31. *Antennospora quadricornuta*. Fig. 26, 27. Ascospores showing gelatinous sheath and subterminal appendages. Fig. 28, 29. Ascospores without sheath. Fig. 30. Immature ascus and spores. Fig. 31. Terminal view of ascospore. Fig. 32–34. *Lignincola laevis*. Fig. 32. Ascospores. Fig. 33, 34. Perithecia. Fig. 1, 2, 30, 33, 34, scale *b*; fig. 8, 9, 12–17, scale *c*; others, scale *a*.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10 μ
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FIG. 1-34

uleana. This fungus is characterized by short, simple conidiophores, each producing a single, acrogenous conidium. The conidia are described as pyriform (obpyriform ?), dark, and muriform (*clathrato-septata*). While there are no figures to substantiate Bubák's description of *P. uleana*, the generic characteristics of *Piricauda* readily include the marine form. *Piricauda pelagica* agrees well with the characteristics (at the generic level) of the type material of *P. uleana* (Hughes, communication). In the former, however, the mature conidia are black as compared with the "dark" (presumably brown) conidia of *P. uleana*. Furthermore, the conidiophores of *P. uleana* are much longer than those occasionally produced by *P. pelagica*. According to Bubák, the foliicolous vegetative mycelium of *P. uleana* is superficial; in *P. pelagica*, the mycelium is both innate and superficial and the fungus is lignicolous.

Helicoma maritimum Linder, *In* Barghoorn & Linder, Farlowia **1**: 405. 1944. (Fig. 49–57). This species is known from a single collection: on a yellow poplar (*Liriodendron tulipifera* L.) panel, submerged 96 days, Intracoastal Waterway, Core Creek Bridge, Carteret County, N. C. This is an estuarine location subject to tidal action. Over a two-year period, salinity at the collection site has varied from 21‰ to 37‰.

The characteristics of the collection vary somewhat from those given by Linder. First, the conidia tend toward more marked coiling (Fig. 55), approaching in this respect, *Helicoma salinum*. Secondly, there are generally more septa in the somewhat larger conidia, and thirdly, the conidial wall is much more deeply constricted at the septa than described for *H. maritimum*. On the other hand, the spreading nature of the colony on wood is clearly analogous to that of Linder's species, *H. maritimum*, and not to *H. salinum*. The latter produces distinct, dense, isolated "patches" on submerged wood; in *H. maritimum* the colony develops over the entire wood surface.

Halosphaeriopsis gen. nov.

Peritheciis innatis, atro-brunneis vel atris, collatis, subglobosis vel ellipsoideis, nonparaphysatis. Ascis irregulariter-clavatis, unitunicatis, deliquescentibus. Ascosporis ellipsoideis, hyalinis, 1–3-septatis, guttulatis, 4-, raro 5-appendiculatis; appendicibus rectis vel curvulis, hyalinis, ex centro radiantibus.

Perithecia innate, dark brown or black, necked, subglobose or ellipsoid, nonparaphysate. Asci 2–8-spored, irregularly clavate, unitunicate, deliquescing. Ascospores ellipsoid, hyaline, 1–3-septate, guttulate; each spore with four, rarely five, stiff, straight or curved, hyaline, setose, equatorial appendages. Type species, *Halosphaeria mediosetigera* Cribb and Cribb, Papers, Univ. Queensland Dept. Bot. **3**(12): 100. 1956.

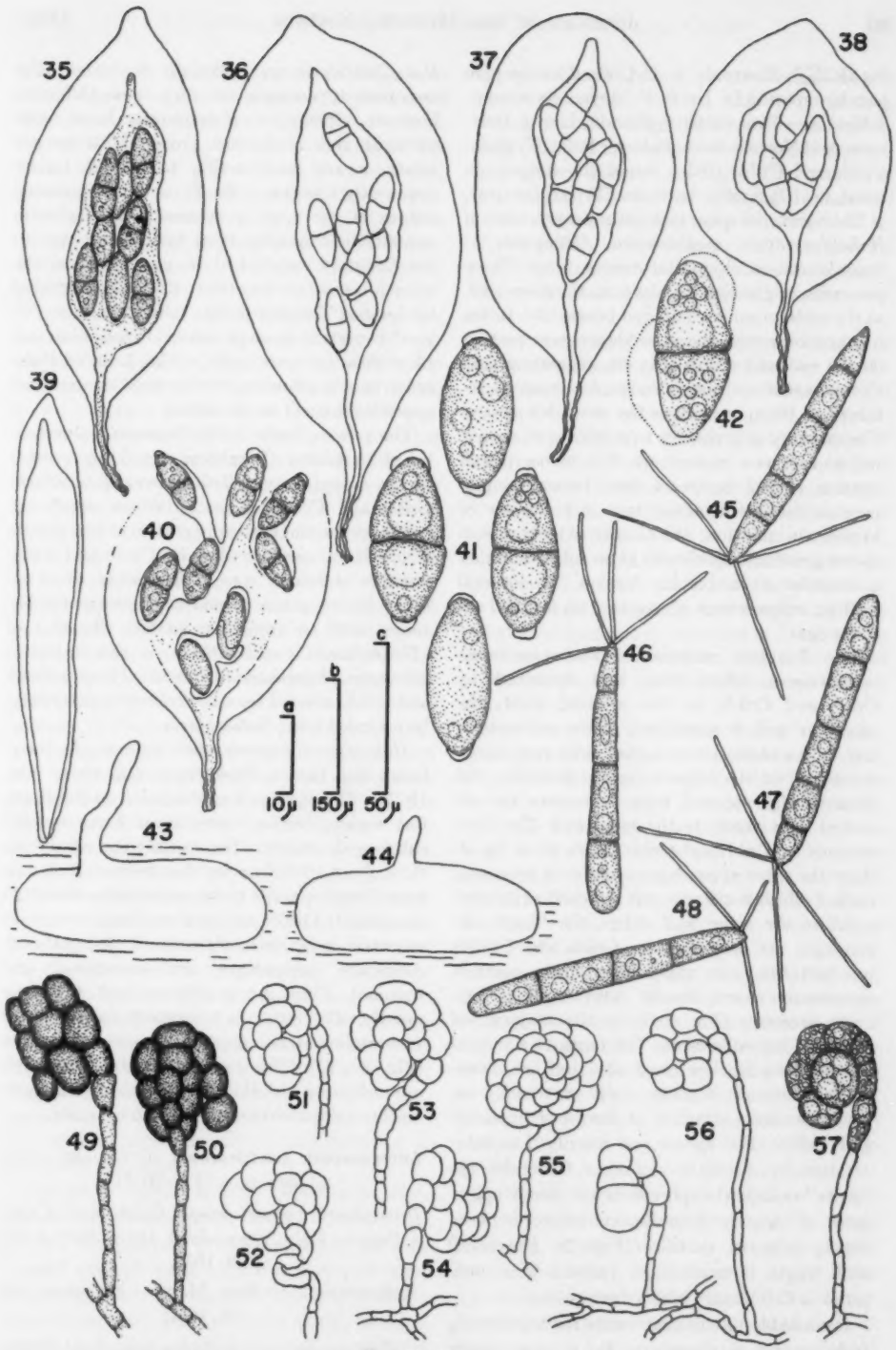
Halosphaeriopsis mediosetigera (Cribb & Cribb) comb. nov. (Fig. 1–9)

Halosphaeria mediosetigera Cribb & Cribb, Papers, Univ. Queensland Dept. Bot. **3**(12): 100. 1956.

Perithecia solitary or gregarious, innate, dark brown or black; subglobose, occasionally ellipsoid; 240–450 μ long by 161–251 μ in diam.; necks occasionally lacking, when present, centric or eccentric, in length, $\frac{1}{8}$ to 3 times the height of the venter, occasionally more than one, cylindric, concolorous; nonparaphysate. Asci irregularly clavate, infrequently irregularly fusiform; unitunicate, apex rounded, base attenuate; usually deliquescing before spores are mature; 2–8-spored; 106–181 × 20–28 μ. Ascospores hyaline, ellipsoid or ellipsoid-fusiform, ends rounded, 1–3-septate; thin-walled, wall constricted at septum; 2–6 guttules in each cell, guttules often pallid yellow; each spore with 4 (rarely 5) slender, hyaline, nonflexuous, curved or straight appendages arising from the equatorial septum; appendages usually equidistant, $\frac{1}{4}$ the length or as long as one cell of the spore, rarely as long as the entire spore; spores occasionally aborting; 25–38 × 8–11 μ, predominantly 28–33 × 8–10 μ.

Four collections of this fungus were made in the Beaufort region: on driftwood dredged from 15 meters, 1½ miles off Bogue Banks; on driftwood, Bird Shoal; on pine panel submerged off Piver's Island, and on yellow poplar panels, submerged 181 days, Neuse River (salinity averag-

FIG. 35–42. *Lentescospora submarina*. Fig. 35–38. Asci, showing thick, gelatinous wall prior to deliquescence. Fig. 39. Immature ascus. Fig. 40. Deliquescing ascus. Fig. 41. Four mature ascospores, showing appendages. Fig. 42. Ascospore with partial gelatinous matrix. Fig. 43–48. *Torpedospora radiata*. Fig. 43, 44. Perithecia. Fig. 45–48. Three- and four-septate ascospores with terminal appendages. Fig. 49–57. *Helicoma maritimum*. Fig. 49. Side view of conidium showing 3-coiled condition. Fig. 50. Mature conidium. Fig. 51–55. Variations in conidia. Fig. 56. Configuration of conidia and conidiophores. Fig. 57. Immature conidium (stippled). Fig. 41, 42, 46–48, scale *a*; fig. 43, 44, scale *b*; others, scale *c*.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b
c
10μ
150μ
50μ

FIG. 35–57

ing 18.5‰), Riverdale, N. C. Collections (on pine panels) reported by Dr. S. P. Meyers (communication) are: Panama City, Florida, Lewes, Delaware, and Norfolk and Gloucester Point, Virginia. Cribb and Cribb (1956) found the fungus, on wood, near Redcliffe, Australia (Type collection).

The equatorial spore appendages or processes of *Halosphaeriopsis mediosetigera* distinguish it from known ascohymenial marine fungi. These processes, originating in pairs from the spore wall, at the midseptum, are stiff and bristle-like. In the few asci observed, the appendages were present on the enclosed spores (Fig. 9), suggesting that the processes are not cytoplasmic remnants retained on the spore wall as the ascus deliquesces. The majority of perithecia were filled with spores, but no asci were in evidence. The ascocarp wall consists of 2–3 layers of dark brown pseudoparenchymatous hyphae; two inner layers of hyphae, in addition, are usually evident. Ascospores germinate by slender germ tubes, in either a unipolar or a bipolar fashion. Monosporal fruiting cultures were propagated on fortified sea water agar.

The Beaufort material of *Halosphaeriopsis mediosetigera* differs from that described by Cribb and Cribb, in two respects. First, the ascospore wall is constricted at the midseptum, and, in 3-septate spores, occasionally very inconspicuously at the adjacent septa. Secondly, the diametrically opposed setose processes are attached individually to the spore wall. The illustrations by Cribb and Cribb (1956, pl. 2, fig. 2) show the spore appendages as pairs of processes, each of which is a single unit attached at its midpoint to the spore wall. Thus, the "four" appendages are, according to Cribb and Cribb's interpretation, two appendages. Phase contrast microscopy shows clearly, however, four separate processes (Fig. 3–6) on the majority of spores of my collections. The bases of a pair of processes are in close proximity, but are, nonetheless, distinctly separate. Only very rarely are the appendages attached as described by Cribb and Cribb. The spores are usually 1-septate; occasionally, 3-septate ones occur. Generally, the "septa" nearest the spore ends are merely aggregates of densely granular cytoplasm between closely adjacent guttules (Fig. 7). Perithecial neck length is much more variable than indicated in Cribb and Cribb's description.

Reasonable justification exists for transferring *Halosphaeria mediosetigera* to a new genus. Ascocarp characteristics of *Halosphaeria* and *Halosphaeriopsis* are essentially the same. The ascospore appendages are very much different, however. Spores of *Halosphaeria* have both terminal and meridional processes; these are gelatinous and flexuous (Fig. 19, 21, 22). Linder apparently recognized the flexuous, deliquescing nature of the spore processes in *Halosphaeria appendiculata* judging from his illustrations. In the Beaufort material of *H. appendiculata*, the spore processes are identical with those illustrated by Linder. The bristle-like, nonflexuous, meridional persistent spore processes of *Halosphaeriopsis mediosetigera* are quite unlike those of *Halosphaeria appendiculata*. Furthermore, no terminal appendages occur in the former.

The generic limits for *Halosphaeria*, as established by Linder (Barghoorn and Linder, 1944) do not entirely admit *Halosphaeria mediosetigera* Cribb and Cribb. If, as I believe, spore appendages are diagnostically critical at the generic level, the species described by Cribb and Cribb must be removed from *Halosphaeria*. Since no other known genus of marine Ascomycetes has spores with an appendage pattern like that of "Halosphaeria" *mediosetigera*, a new taxon is necessary. *Halosphaeria trifurcata* (Höhnk) Cribb and Cribb, which I have not collected, should also be excluded from *Halosphaeria*.

Halosphaeria appendiculata Lindner, *In* Barghoorn and Linder, Farlowia **1**: 412. 1944. (Fig. 19–25). This species is not common in the Beaufort region, having been found twice on 187 submerged panels. The perithecia are brown throughout, differing in this respect from the type. Moreover, 2–4 necks occasionally occur (on basswood); Linder reported a single, centric or eccentric neck on perithecia of his material. Ascospore appendages are meridional and terminal. They are gelatinous and deliquesce rapidly. The latter fact accounts for fewer (or very rudimentary) appendages on some spores (Fig. 20, 25). The relationship of *Halosphaeria appendiculata* and *Halosphaeriopsis mediosetigera* has been discussed under the latter species.

Antennospora quadricornuta (Cribb and Cribb comb. nov. (Fig. 26–31)

Halosphaeria quadricornuta Cribb and Cribb, Papers, Univ. Queensland Dept. Bot. **3**(12): 99. 1956.

Antennospora caribbea Meyers, Mycologia **49**: 503. 1957.

This species has a confusing taxonomic history. Meyers (1954) reported the fungus as Form 2,

but did not, at the time, name it. In 1957, however, Meyers applied the name *Antennospora caribbea*, a new genus and species, to Form 2. Meanwhile, Cribb and Cribb (1956) found the same fungus and placed it as a new species, *quadricornuta*, in Linder's genus *Halosphaeria*. The principal issue involved in selection of a proper category for the fungus concerns interpretation of the nature of ascospore appendages in *Halosphaeria*.

Cribb and Cribb (1956) assigned four species to the genus *Halosphaeria*, including *H. appendiculata* Linder. Concerning this disposition of species, Cribb and Cribb wrote, in part (1956, p. 99): "In each the spores are hyaline and 1-septate or 1–3-septate, with slender hyaline tapering appendages of similar nature, variously disposed. As far as can be seen, the appendages are of the same type as in *H. appendiculata*. The appendages in all four species appear rigid when the spores are mounted in sea water, but may become lax in lactophenol, appearing as shown in Linder's figure." Certainly, there is a marked difference in the nature of appendages in *Halosphaeria* (*Halosphaeriopsis*) *mediosetigera* and *H. appendiculata*, as has previously been noted. The same may be said for *Halosphaeria quadricornuta*. In this species, the appendages are stiff, and nongelatinous, whereas in *H. appendiculata* the appendages are flexuous and gelatinous. Meyers was correct in assigning his fungus to a new genus, *Antennospora;* the specific name used by Cribb and Cribb must stand, however, since it antedates the name selected by Meyers.

The Beaufort material of *Antennospora quadricornuta* differs from that described by Cribb and Cribb and by Meyers principally in the fact that the ascospore wall is conspicuously constricted at the midseptum. The aforementioned workers specified clearly that the spore wall was not constricted. Furthermore, the spores are occasionally furnished with a gelatinous sheath (Fig. 26, 27). The sheath is rapidly deliquescent in sea water, suggesting that it is secondary in nature and not formed as a part of the spore as it is delimited in the developing ascus.

Lignincola laevis Höhnk, Veröffentl. Inst. Meeresfors. Bremerhaven **3**: 216. 1955. (Fig. 32–34). This species occurs commonly on submerged wood in marine habitats, and very commonly on wood in brackish water. *Lignincola laevis* is one of the few lignicolous marine ascomycetous fungi which do not produce appendaged ascospores. The spores of *L. laevis* are cerio-sporopsis-like in appearance—two celled, slightly constricted at the septum, and, when mature, having a single, large, pallid yellow guttule in each cell. The spores lack the terminal appendages of *Ceriosporopsis*, however. The material of this fungus collected on submerged basswood (*Tilia americana*) differs from that described by Höhnk (1955), and by Meyers (1957), in that the perithecia are small and short-necked; a single, long-cylindrical neck is apparently characteristic of *L. laevis*.

Lentescospora submarina Linder, *In* Barghoorn and Linder, Farlowia **1**: 411. 1944. (Fig. 35–42). This species is known in the Beaufort Inlet region, from two collections: on pine panels submerged 181 days in 7 meters (mean low tide) of water at 27.8–36.1‰ salinity, and on yellow poplar panels (*Liriodendron tulipifera*) submerged 63 days in 4 meters (mean low tide) of water at 7.3–18.2‰ salinity. Both collections agree remarkably well with the characteristics given by Linder. Asci, however, have never been described for the monotypic genus *Lentescospora*. I have found abundant asci in ascocarps of *L. submarina* collected from the mesohaline area. A brief note on the morphology and deliquescence of the asci seems justifiable.

Immature asci containing densely granular, homogeneous cytoplasm, are thick walled with a distinct apical and basal canal (Fig. 39). As the spores are delimited and approach maturity, the ascus wall expands perceptibly except in the basal portion. Asci containing spore initials are ovoid or ellipsoid, attenuated basally, and often apiculate (Fig. 35, 36, 38). The hyaline ascus is bitunicate. The inner wall, when expanded, is very thick, averaging 73 μ. Both the endo- and ectoascus deliquesce rapidly, suggesting that both walls are gelatinous. No cellulose reaction occurs when the asci are treated with chloro-iodide of zinc. The basal canal of the expanded ascus extends to the ascus base; the apical canal does not reach the ascus apex, however (Fig. 35). Ascospores are arranged irregularly or in a compact group in the lumen, but do not fill it (Fig. 36, 38).

Perithecia commonly have only four or five asci, but often, numerous "free" spores. Some perithecia, on the other hand, contain only ascospores. The ostiole is conspicuous in the latter perithecia but very inconspicuous in the former. These observations suggest, first, that the asci swell upon the influx of water into the perithecial venter, and secondly, that the asci are produced

successively, deliquesce, and liberate the spores within the venter. Moreover, spore discharge is likely coincident with breakdown of the cells within the ostiole, and not with maturation of the asci, such as in *Leptosphaeria discors* (Johnson, 1956b). Such a hypothesis would account for the few asci observed at any one time in a given perithecium, and the numerous spores which fill the mature fruiting body. I have not been able to observe this directly.

The ascus opens to liberate spores by deliquescence of the gelatinous wall (Fig. 40). Asci crushed from the perithecium into the surrounding water deliquesce within three minutes, with the basal stalk being the last to disintegrate. As deliquescence proceeds the spores separate, yet are clearly attached by a hyaline, gelatinous material which persists for 10–15 minutes (Fig. 40, 42). The matrix around each spore gradually dissolves except for a small portion on the ends of each spore; this portion is the gelatinous "cap" characteristic of the mature spore (Fig. 41). Thus, the appendages of *Lentescospora submarina* are very likely secondary in nature, and represent remnants of a matrix around the developing spore.

Lentescospora submarina is closely allied to *Ceriosporopsis halima*, differing principally in the nature of the terminal spore appendages. In *L. submarina*, these are hyaline, gelatinized, terminal "caps", while in *C. halima*, the spore ends terminate in a gelatinous, hyaline, attenuated appendage. Ascospores of *C. halima*, particularly from perithecia which develop on submerged wood in an oligohaline environment, often have very much shortened appendages that approach, in general configuration, the hyaline "caps" of *L. submarina* (unpublished notes). This suggests that intergrades occur between these two genera. Furthermore, the asci of *C. halima*, like those of *L. submarina*, are bitunicate with a thin extensile ectoascus and a gelatinous endoascus. These two species are unquestionably more closely related than previous observations suggest.

Torpedospora radiata Meyers, Mycologia **49**: 496. 1957. (Fig. 43–48). Two collections of this fungus have been made in the Beaufort region. In both, the ascocarps are very sparse. Meyers (1954, 1957) reports globose perithecia for his material, both on the original wood substratum and in culture. In the Beaufort material, the ascocarps are elongate or depressed globose on wood (basswood). Furthermore, the ascospores are often 5-celled (4-septate) as compared with 4-celled (3-septate) spores described by Meyers. Minor differences between the type and my material appear in spore length and in appendage diameter.

SUMMARY

Seven lignicolous marine fungi are reported from the Beaufort Inlet region, North Carolina. Among the Fungi Imperfecti, *Piricauda pelagica* is described as a new species. *Halosphaeria mediosetigera* is placed in the new genus *Halosphaeriopsis*. *Antennospora quadricornuta* is proposed as a new combination for *Halosphaeria quadricornuta* and *Antennospora caribbea*. The asci of *Lentescospora submarina* are described.

ACKNOWLEDGMENTS

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MORPHOLOGY AND CYTOLOGY OF THE ZOOSPORANGIA AND CYSTOSORI OF *SOROSPHAERA VERONICAE*

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The cystosori of *Sorosphaera veronicae* which occur so commonly in enlargements of the shoot system of *Veronica* have been known since their discovery by Schroeter in 1897. In the mycological laboratory at Chapel Hill this parasite, which occurs abundantly close to the botany building, has been used for many years in the mycology classes. In this laboratory thorough searches were made for zoosporangial thalli, but without success, until in February, 1956, for the first time entire host plants were brought into the laboratory and, after the root systems had been washed, they were examined for zoosporangial thalli. In these roots zoosporangial thalli of a plasmodiophoraceous organism as well as zoosporangia of a chytrid were found. Could these zoosporangial thalli be the long sought sporangial stage of *Sorosphaera*? It was in the hope of throwing some light on this problem that this study was undertaken.

Zoosporangia have been found in all true members of the Plasmodiophoraceae except *Tetramyxa* and *Sorosphaera*, in which only the cystosori are known. This paper contains an announcement of the discovery of the zoosporangia of *Sorosphaera veronicae*, with a description of their morphology and cytology, and some new observations on the cystosori.

Morphology and Life History Studies on Living Material

Initial observations, experiments and results.— The initial stages of this study were confused by the several apparently different stages in parasite development which were found. Plasmodia and cystosori typical of *Sorosphaera veronicae* were present in the galls produced on the shoots of the host. Cystosori and sporangiosori which seemed to belong to the genus *Ligniera* or possibly *Polymyxa* were found in the roots. In addition, relatively giant sporangia-like structures with large discharge apertures were present as well as small star-shaped resting thalli and spherical, thin-walled sporangia each with or without a single tube-like discharge pore. With further study it became clear that there were two plasmodiophoraceous parasites present in the roots of *Veronica persica* and *V. hederaefolia: Ligniera verrucosa*, including both the cystosori and the previously unknown sporangial stage which is to be described in a later paper; and the giant sporangia mentioned above. The small star-shaped resting thalli and spherical thin-walled sporangia were found to be *Asterocystis radicis*, an olpidiaceous parasite of the roots of some crop plants. The large plasmodiophoraceous sporangia were assumed to be the heretofore unknown sporangia of *Sorosphaera veronicae*.

Veronica persica, because of the very large number of seeds which it produces, was used exclusively as the host in the host-parasite cultural studies. Seeds and galls from the aerial portions of this plant, which begins growth in October and November, flowers during December through April, and fruits in April and May, were collected in large quantities. During April, 1956, attempts to germinate freshly collected seeds of the host plant, so that cultural studies could be started, were unsuccessful. This was not surprising, for as Crocker and Barton (1953) point out, weed seeds remain dormant and viable for long periods of time in the soil, until physical and chemical changes have taken place. Attempts were made to break this dormancy. Scarification, sulfuric acid and boiling water treatments of seeds did not prevail, for no seeds germinated. Seeds were washed with slowly running water to remove possible germination inhibiting substances. Experiments whereby after-ripening changes in these seeds could be sped up were started. Some seeds were placed in a moist condition at a temperature of 2°–4°C.; alternating temperatures of 15°–30°C., sixteen hours in the

[1] This paper is part of a dissertation submitted to the Faculty of the University of North Carolina in partial fulfillment of the requirements for the Ph.D. degree in the Department of Botany. I would like to express my thanks to Dr. John N. Couch, under whose direction this work was carried out, for suggesting this problem, and for helpful criticisms and suggestions throughout the study.

former and eight hours in the latter, were tried. Under none of the conditions described above did germination occur, and the seeds were then stored in the refrigator at 2°–4°C., dry, in hopes that low temperature and time might aid in breaking dormancy.

Six months later some of these seeds were taken out of the refrigerator and placed in petri dishes on moist filter paper at room temperature. After one week only four of fifty seeds had germinated. These same dishes containing the ungerminated seeds on moist filter paper were now returned to the refrigerator. Nine days later, ninety per cent of these seeds germinated when they were returned to room temperature. Repeated experiments indicated that generally six days in a moist condition at 2°–4°C. after the initial wetting would give high percentages of germination.

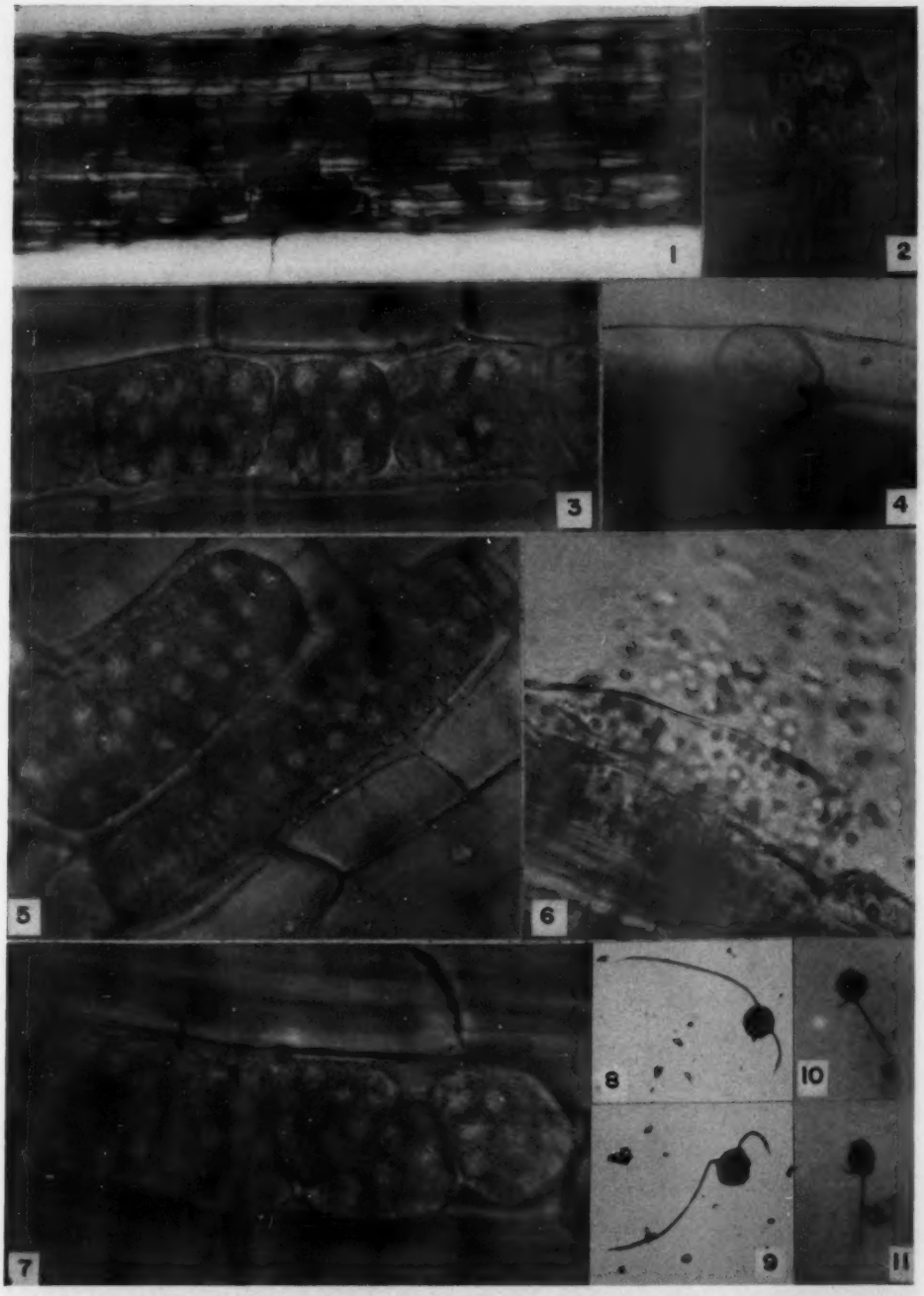
Now that the seeds would germinate, plans to grow and infect the seedlings with the parasite could be realized. Soil was obtained from one of the areas in which the host plant and the parasite were found. This soil was spread out in large stainless steel trays and autoclaved for thirty minutes at fifteen pounds pressure on three successive days. The sterile soil was then placed in new clay pots, and these were autoclaved at fifteen pounds pressure for fifteen minutes. Into each of the six resulting pots were planted several young seedlings of *V. persica*. These plants were grown in the algal culture room at temperatures and periods and intensities of light which simulated the external environmental conditions under which the host plant, a winter annual in this part of the United States, thrives.

The zoosporangia of this parasite were collected by first searching for host plants bearing galls on their shoot systems. When a desired specimen was found, an area of soil surrounding the plant, deep enough to include the terminal portions of the roots, was dug up. After this material had been brought back to the laboratory, it was washed with cold tap water to remove all the soil; at the same time other roots and debris present were removed. The root system thus freed from external foreign matter was then placed in ice water so that sporangial discharge, which occurred best in cold water, was promoted. Unlike the cystosori, which produce distinct galls on the shoot, the zoosporangial thalli produce no external symptoms such as hypertrophy or discoloration by which their presence may be detected with the unaided eye. It was necessary therefore to cut small bits off the ends of the root system which were then mounted on slides for viewing with the compound microscope, or a search was made with a dissecting microscope for heavily infested roots and these were cut from the plant. Zoosporangia thus collected were placed in small dishes of cold water and allowed to discharge their spores. This water containing swimming zoospores was used in the experiments described below.

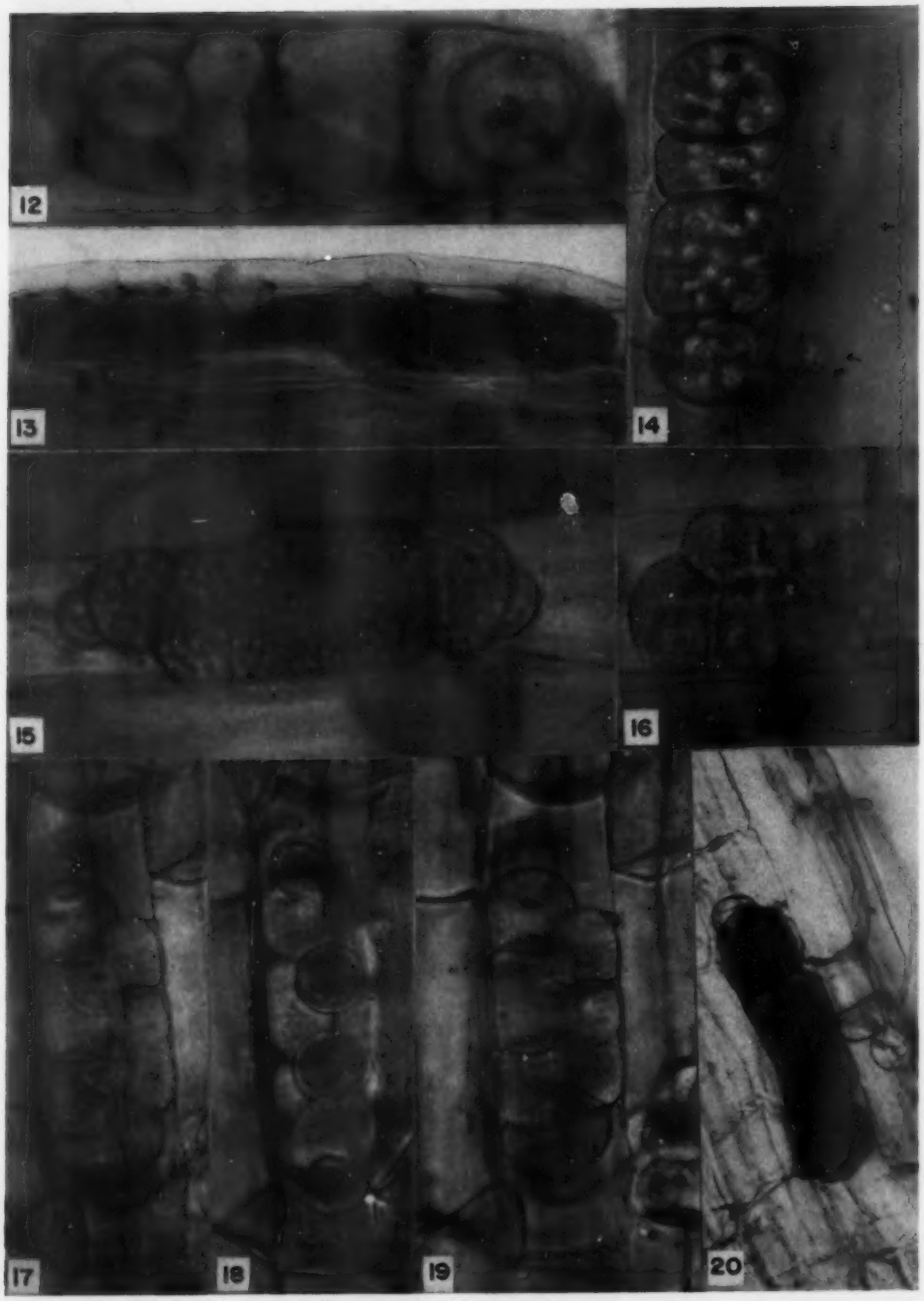
Ligniera verrucosa was occasionally found parasitizing the same host plant. This parasite completes its life cycle in the epidermal and subepidermal cells of the roots of the host. Unlike *Sorosphaera veronicae*, which forms zoosporangia in the roots and cystosori in the shoot system, *L. verrucosa* forms both structures in the roots. Also the zoosporangia of *S. veronicae* have never been found in root hairs, but both zoosporangia and cystosori of *L. verrucosa* occur in root hairs as well as in unmodified epidermal cells. In selecting host roots infested with zoosporangia to be used for the collection of zoospores of *S. veronicae*, efforts were made to exclude those roots which were found to contain zoosporangia and/or cystosori of *L. verrucosa*. Because of this, probably, zoosporangia or cystosori of *L. verrucosa* were never found in any of the cells of the experimental plants.

Two of three pots with host plant seedlings as much alike as possible growing in them were flooded several times with water containing swimming zoospores. As a result of this, the seedlings were often covered with water for several minutes. The third pot, the control, was watered with distilled water. One week later another experiment exactly as above was set up, making a total of four pots which had zoospores added to them. Approximately two months later, the roots of the plants in three of the four pots were found to be heavily parasitized by the plasmodia and zoosporangia of *S. veronicae*. In addition one plant produced a relatively large gall in the axil of the cotyledon. This gall contained typical cystosori of *S. veronicae*. The plants in the two control pots showed no evidence of plasmodia, zoosporangia or cystosori. All the plants in the above experiments were flooded with distilled water every day, and they were watered with a complete nutrient solution (Greulach, 1952) once a week.

Because the soil as used in the above two experiments contained large amounts of organic matter which tended to support contaminating



FIGS. 1–11. *Sorosphaera veronicae* in root cells of *Veronica persica*. Figs. 1–7 from living material. Fig. 1. Host cells infected with plasmodia and zoosporangia. ×176. Fig. 2. Young plasmodium showing nuclei in interphase. ×819. Fig. 3. Young plasmodia. ×819. Fig. 4. Enlargement of a single discharge pore of sporangia pictured in fig. 13. ×1088. Fig. 5. Large multinucleate plasmodia. ×819. Fig. 6. Discharge of zoospores. ×819. Fig. 7. Five plasmodia in a single cell. ×1229. Figs. 8, 9. Biflagellate heterocont zoospores from zoosporangia killed and fixed over 2 per cent osmic acid fumes and stained using Löffler technique. Note whip-lashes on both flagella. ×1088. Figs. 10, 11. Biflagellate heterocont zoospores from resting spores of cystosori stained with crystal violet. ×1135.



FIGS. 12-20

fungi such as *Penicillium* and *Rhizopus*, it was decided to use sand and a complete nutrient solution as a substrate in future repeat experiments. Therefore, 30 mesh pure quartz sand was washed for 24 hours in running tap water and sterilized in an oven at 270°C. for 24 hours. This sand was then placed in new clay pots, and two of these pots were then placed in a plastic container which held the supply of nutrient solution.

Veronica persica seedlings were planted in pots as prepared above. Into two of these pots, cold water containing swimming zoospores of the parasite was poured. Finely ground galls of the host were sprinkled on the surface of the sand of two other pots. Approximately every five days the remaining nutrient solution was poured off and a fresh supply added to each container. At the end of one month the plants were removed from the pots and examined. In the experiment where zoospores were added to the young seedlings, almost all of the plants were found to have large numbers of plasmodia and zoosporangia in their roots. In addition, two plants, one in each of the experimental pots, produced severely hypertrophied shoot systems which were found to be galls containing typical cystosori of *Sorosphaera veronicae*. The roots of the plants in the other two pots to which had been added finely ground galls were found to contain large numbers of plasmodia and zoosporangia of the parasite. The experiment described above where finely ground galls containing vast numbers of *S. veronicae* cystosori were sprinkled on young host plant seedlings was repreated two more times. The gall material used in all experiments of this type had been stored dry for ten to twelve months before it was ground. Every experimental plant in all four pots of both experiments was found to have abundant plasmodia and zoosporangia in its root system. In all experiments reported above the control plants never showed any evidence of parasite infection.

Additional evidence which supports the premise that the sporangia and plasmodia found only in the roots of *V. persica* and the cystosori found only on the shoot system are part of the life cycle of *S. veronicae* was obtained from a survey of host plants from widely separated areas on the campus. Whenever galls were found on the shoot systems of the host plants, plasmodia and zoosporangia were found in the root systems. Also, the zoospores of cystosori and zoosporangia are biflagellate and heterocont, typical of those found in the Plasmodiophoraceae (Figs. 8–11), and cytological studies reported below indicate that both phases are plasmodiophoraceous parasites.

Sorosphaera veronicae was described as a parasite on *Veronica hederaefolia*, *V. chamaedrys* and *V. triphyllos* by Schroeter. Since that time it has been found parasitizing ten other species of this genus by various workers. On the campus of the University of North Carolina, *S. veronicae* has been observed parasitizing *V. hederaefolia*, *V. persica* and *V. peregrina*, the latter two being newly reported hosts.

Morphology and Development of Sporangia.—The zoosporangium of *Sorosphaera veronicae* begins its development in the epidermal cells, root hairs excepted, and cortical cells of the roots of *Veronica* as a uninucleate, naked thallus (Fig. 85). This thallus grows and eventually becomes a naked multinucleate protoplast usually referred to as a plasmodium (Figs. 1–3, 5, 7, 16, 86–89, 92). The heavily granular cytoplasm of the living plasmodium surrounds relatively clear areas, which are the nuclei of the thalus. Eventually the plasmodium ceases growth, and it may become lobed or segmented into segments of various sizes (Fig. 1). Exactly how this process occurs has not been determined. Walls are eventually formed around these multinucleate segments (Figs. 13, 15, 19, 20, 90, 91). However, each segment apparently remains connected with adjacent ones, for the zoospores seem to be able to swim without hindrance from one segment to

FIGS. 12–20. *Sorosphaera veronicae* in root cells of *Veronica persica*. All Figs. except 13 and 20 from lining material. Fig. 12. Empty zoosporangia. ×1088. Fig. 13. Zoosporangia with discharge pores seen in side view. ×503. Fig. 14. Discharging zoosporangia. Zoospores swarming in sporangia; some have escaped (see to right of sporangia). ×819. Fig. 15. Zoosporangia with zoospore initials. ×754. Fig. 16. Young plasmodia. ×819. Figs. 17–19. The same sporangia photographed at different focal levels of the microscope. ×754. Fig. 17. Double exposed photograph showing the focal level as seen in fig. 18 superimposed on the focal level as seen in fig. 19. Fig. 18. Top view of discharge pores, taken at higher focal level. Fig. 19. Photograph taken at a lower focal level so that discharge pores are not seen. Fig. 20. Undischarged zoosporangia on left; empty zoosporangia to the right. ×503.

another. Also several segments may share a single discharge pore (Figs. 17, 18, 19, 20).

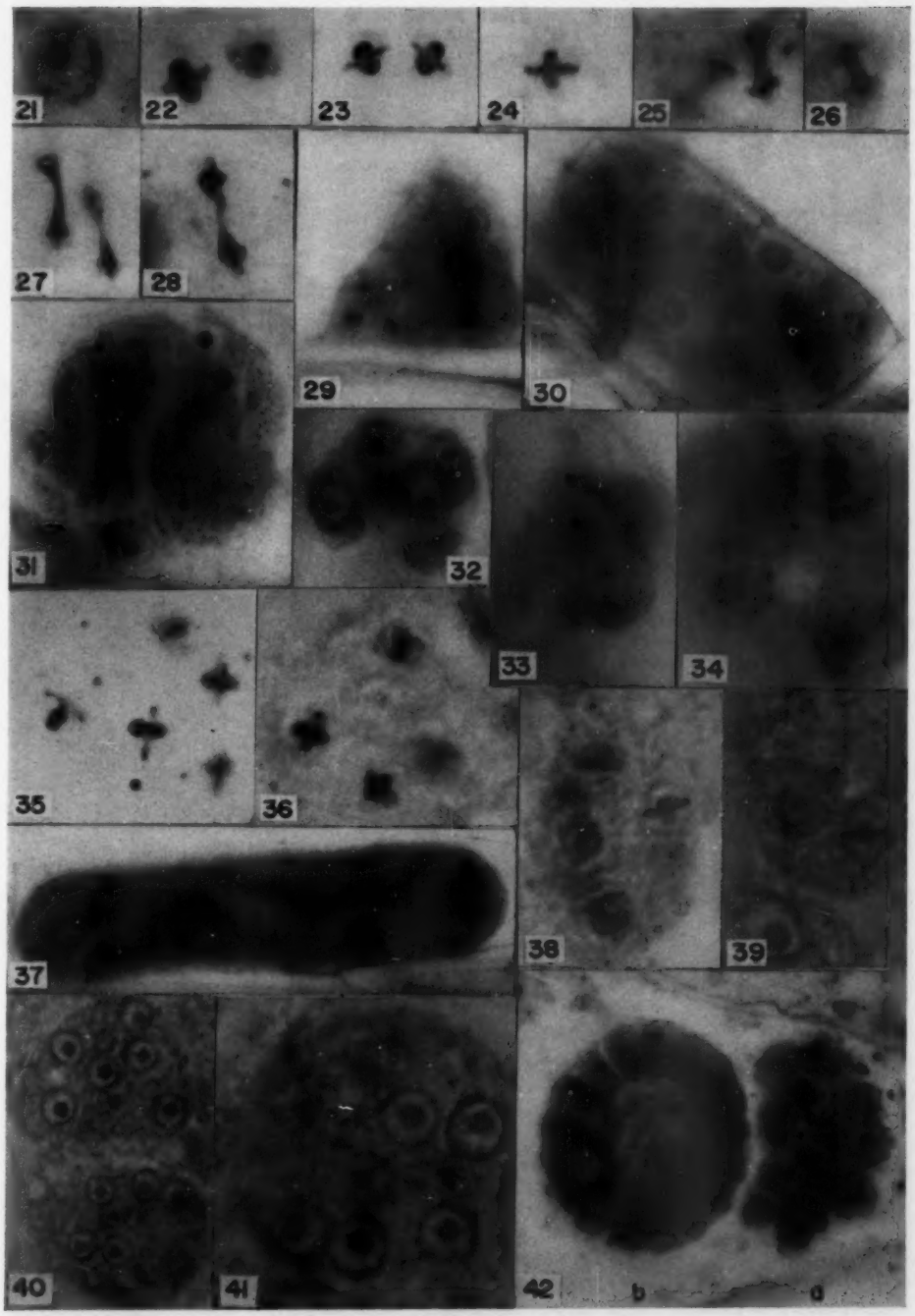
Actual penetration of the host cells by the infecting zoospore has not been seen, although many zoospores which had encysted on epidermal cells of fresh, young *V. persica* seedlings were watched for long periods of time. Empty cysts were not observed on the surface of host cells. Possibly these could have been washed away during the preparation of the material for study. On the other hand, Ledingham (1939) also found no empty cysts on the surface of root hairs after penetration by the zoospore of *Polymyxa graminis*. He suggested that possibly, as was found by Curtis (1921) for *Synchytrium endobioticum*, the entire amoeba passes into the cell.

The terms sporangiosorus and sporangium are useful in designating zoospore-producing structures in fungi. Usually the sporangiosorus is an aggregation or sorus of sporangia, each sporangium producing its own discharge pore as found for example in the Synchytriaceae. However, in some of the Plasmodiophoraceae several zoosporangia may be connected by narrow isthmuses as was reported by Couch, Leitner and Whiffen (1939) in *Octomyxa achlyae*. Goldie-Smith (1951, 1954) reports for *Sorodiscus cokeri* and *Woronina polycystis* and Couch, Leitner and Whiffen (1939) for *Octomyxa achlyae* that sporangia in the interior of the sorus do not produce exit tubes but that their zoospores escape by passing from one sporangium to another "through narrow pores that develop in contiguous walls" (Goldie-Smith, 1951). In *Polymyxa graminis*, a genus erected by Ledingham (1939) because the zoosporangial characteristics of this organism differed from those of other genera of the Plasmodiophoraceae, large septate zoosporangia are found. Each multinucleate segment or lobe of these unusually large sporangia has one or more discharge tubes. The zoospores are not able to pass from one segment to another. Thus in this group of organisms it is sometimes not easy to determine the limits of a sporangium.

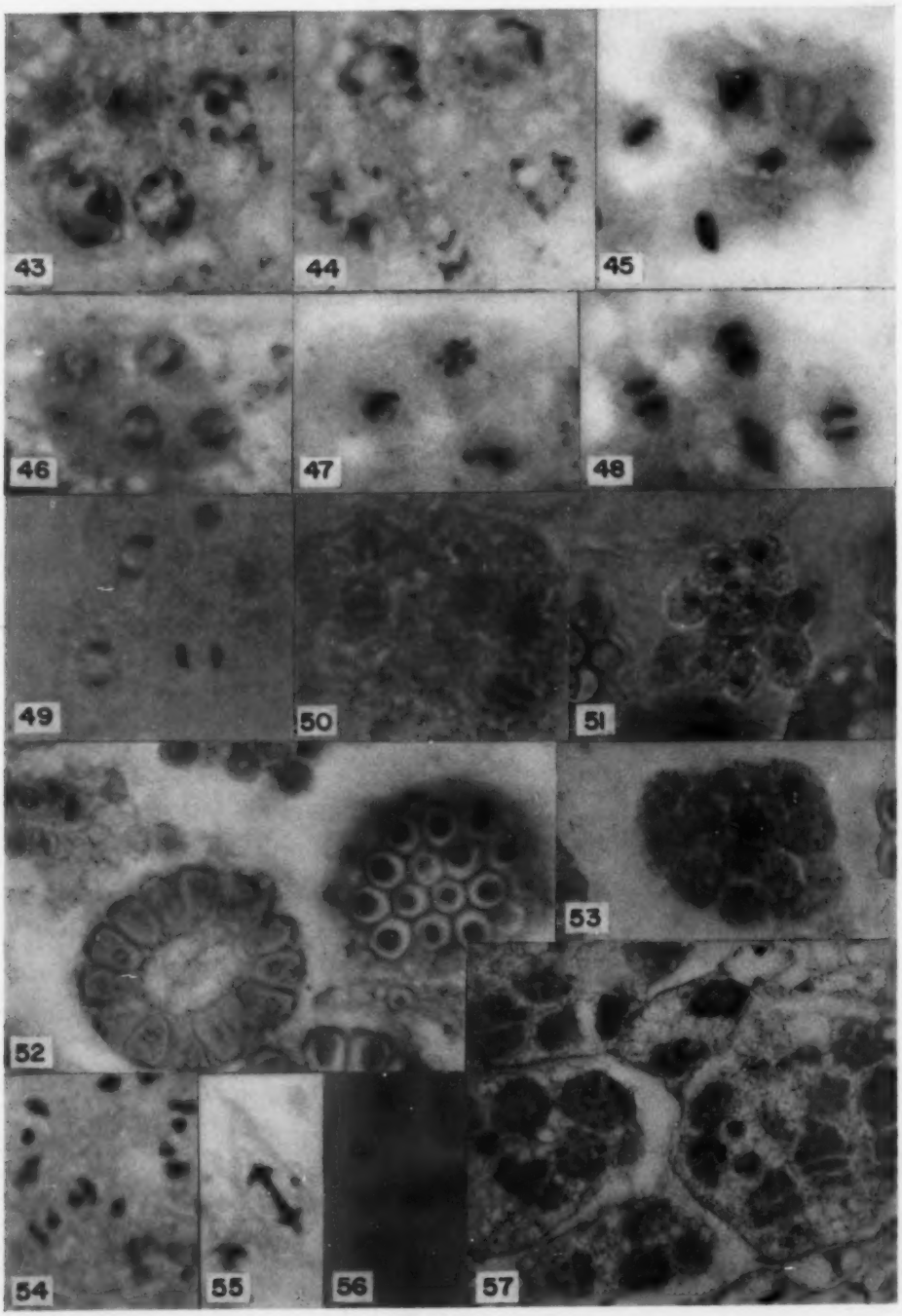
In *Sorosphaera veronicae* this problem seems especially perplexing. Here the compound structure (sporangiosorus ?) appears to be composed of several distinct units or segments (sporangia ?) (Figs. 12, 14, 15, 17–20, 90, 91). However, the septa of these apparently distinct segments do not prevent zoospores from swimming to adjacent units. Often these units share a common discharge pore, and they have always been observed to be in the same stage of development (Figs. 12, 14, 15, 19, 20). It seems necessary to consider if a compound structure composed of units connected by isthmuses or pores is truly a sorus of sporangia. Should it be considered a single lobed or catenulate sporangium? It appears then that there may be a variety of sporangial expressions in the Plasmodiophoraceae, and as Ledingham points out, in this respect this group of organisms is not unique.

The discharge papilla is large, having generally a bulbous shape (Figs. 4, 13, 18, 90, 91). The very large aperture provides in most cases a passage for escape of zoospores from different units of the same sporangium. The escape of zoospores may be made possible by dissolution of the host cell wall at points where the discharge papilla comes in contact with it, or as Ledingham (1935) observed in *Spongospora subterranea*, the pressure on the host cell wall which results from these bulbous swellings may rupture it. The size and

FIGS. 21–28. Stages in cruciform division in zoosporangial plasmodia. Stained with crystal violet. Fig. 21. Interphase nucleus. ×2425. Fig. 22. Cruciform stage, one figure in polar view. ×2910. Fig. 23. Cruciform stage. ×2790. Fig. 24. Cruciform stage. Note chromosomes in ring across the nucleolus. ×2700. Figs. 25, 26. Dumbbell or double anchor stages. ×2910. Fig. 27. Telophase stage. Nucleolus has begun to constrict. ×2910. Fig. 28. Final constriction of nucleolus. ×2910. Figs. 29–36 stained with crystal violet. Fig. 29. Young zoosporangial plasmodia. ×1395. Fig. 30. Developing zoosporangium. ×1455. Fig. 31. Multinucleate zoosporangial plasmodia. One in double anchor stage of division. ×1395. Fig. 32. Young plasmodial thalli in root cells. ×1455. Fig. 33. Young zoosporangial plasmodia. ×1455. Fig. 34. Interphase nuclei of zoosporangial plasmodium. ×2910. Fig. 35. Cruciform stage of cruciform division in cystosoral plasmodia. ×2910. Fig. 36. Cruciform stage of division in cystosoral plasmodia viewed obliquely. Note discontinuous ring of chromosomes. ×2910. Fig. 37. Large multinucleate plasmodium in epidermal cell of the roots. Mounted in Amann's Lacto-phenol plus acid fuchsin. ×970. Figs. 38, 39. Cruciform stages in cruciform division in cystosoral plasmodia. Stained using Feulgen technique and light green. ×2910. Fig. 40. Multinucleate cystosoral plasmodia. Stained using Feulgen technique and light green. ×1455. Fig. 41. Interphase nuclei of cystosoral plasmodium. Stained using Feulgen technique and light green. ×2910. Fig. 42a. Incipient resting spores. Stained with crystal violet. ×1455. Fig. 42b. Young cystosorus. Stained with crystal violet. ×1455.



FIGS. 21-42



FIGS. 43-57

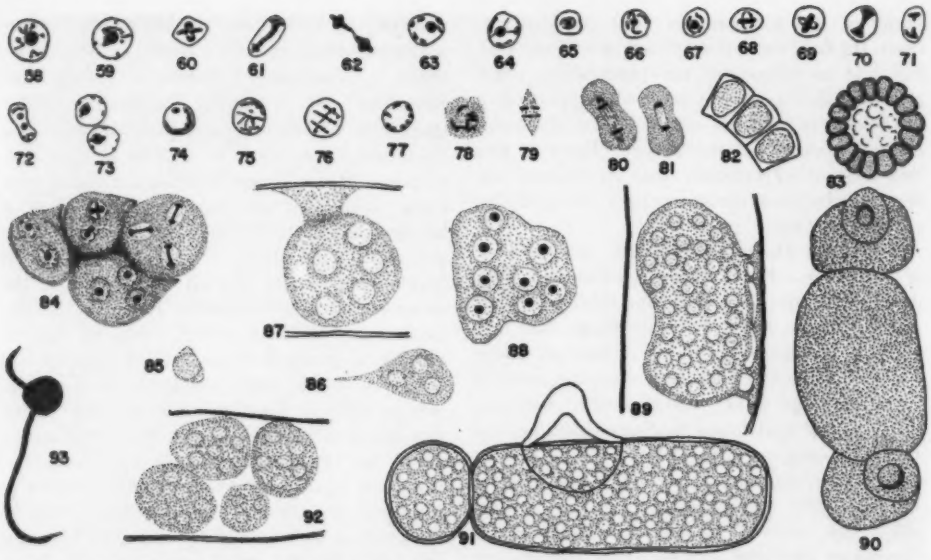
shape of the zoosporangia vary considerably. Often the final size is determined by the host cell, but just as frequently the plasmodium stops growing before filling the host cell. Hypertrophy of the cells of the roots caused by the plasmodia or zoosporangia has never been observed, and this lack of hypertrophy may be one of the reasons why these structures have not been discovered previously.

Sporangial Discharge, Structure and Behavior of Zoospores.—The zoospores, which are fully developed in the zoosporangium, exhibit a gentle rocking motion just prior to discharge. The first spore to escape seems to be forced out under pressure while the ones later escaping actually swim out (Figs. 6, 14). The method of discharge is much like that found in *Saprolegnia*, i.e. the first zoospores, expelled under pressure, one by one, remain quiescent, just barely rocking, for approximately thirty seconds, and then with a slight jerk, swim off clumsily and erratically. If conditions are kept suitable, cool temperature being the most important, the swimming becomes quite rapid and more graceful. At discharge and as long as it swims, the zoospore is bulged in the center with pointed ends. Possibly because conditions become unsuitable, the zoospore begins to encyst. As this happens the spore body becomes spherical, and the long posterior flagellum is dragged along behind as the anterior flagellum lashes slowly back and forth. Swimming in this relatively inactive manner, the zoospore may rotate in a circle. Eventually it encysts. No certain examples of amoeboid zoospores were observed.

Prior to the work of Ledingham (1934, 1935, 1939) the zoospores of the Plasmodiophoraceae were reported by all observers as posteriorly uniflagellate. Since that time biflagellate heterocont zoospores have been reported in *Plasmodiophora*, *Spongospora*, *Octomyxa*, *Polymyxa*, *Sorodiscus* and *Woronina*. Ledingham (1935) figured the flagella of *Spongospora subterranea* as thick and blunt-ended. In 1939 he reported the flagella of the zoospores of *Polymyxa graminis* as being blunt-ended also, but close observation of his figure 25, Plate III, shows a whip-lash on the end of each flagellum. Ellison (1945) reported blunt-ended flagella without whip-lashes on the zoospores of *Plasmodiophora brassicae*. Couch, Leitner and Whiffen (1939, plate 48, fig. 3) include a photomicrograph of a zoospore of *Octomyxa achlyae* which appears to have a whip-lash on the long flagellum while the short flagellum apparently lacks the whip-lash. Pendergrass (1950, fig. 14) includes a figure of a zoospore of *Octomyxa brevilegniae* which seems to have a whip-lash on the posterior flagellum, and he reports that no tinsels were observed after using the Löffler flagellar staining technique. Dr. W. J. Koch (unpublished research) found whip-lashes on both flagella of the zoospores of *Woronina glomerata*, which he found parasitizing the alga *Vaucheria*. Goldie-Smith (1951) reports biflagellate heterocont zoospores in *Sorodiscus cokeri*, but her figures do not indicate, nor does she report, that whip-lashes are present on the flagella. In 1954 Goldie-Smith reported receiving a communication from Dr. J. T. Barrett which included a photomicrograph showing that each flagellum of the zoospores of *Woronina polycystis* ended in a whip-lash, and she further included in this paper photomicrographs which show the zoospores of her isolate of *W. polycystis* as having a whip-lash on each flagellum.

In the light of the above discussion, and since

FIG. 43. Early prophase of first sporogenic division of cystosoral plasmodia. Stained with crystal violet. ×2910. Fig. 44. Late prophase of same; nucleolus has disappeared. Stained with crystal violet. ×2910. Fig. 45. Metaphase of same. Stained with crystal violet. ×2910. Fig. 46. Prophase (?) of same. Stained with crystal violet. ×1940. Fig. 47. Metaphase of same. Polar view. Stained using Feulgen technique and light green. ×2910. Fig. 48. Anaphase of same. Stained with crystal violet. ×2910. Fig. 49. Telophase of second sporogenic division. Stained using Feulgen technique and light green. ×2910. Fig. 50. Beginning anaphase or so-called double anchor or dumbbell stage of cruciform division in cystosoral plasmodia. Stained using Feulgen technique and light green. ×2910. Fig. 51. Metaphase of second sporogenic division. Stained using Feulgen technique and light green. ×1455. Fig. 52. Incipient resting spores and cystosori. Stained using Feulgen technique and light green. ×1455. Fig. 53. Incipient resting spores. Stained using Feulgen technique and light green. ×1455. Fig. 54. Stage of cruciform division in cystosoral plasmodia just prior to division of nuclear membrane. Stained with crystal violet. ×2910. Fig. 55. Double anchor stage of cruciform division in cystosoral plasmodia. Stained with crystal violet. ×2910. Fig. 56. Telophase of second sporogenic division. Stained with crystal violet. ×2910. Fig. 57. Cross section view of gall of *V. persica* showing host cells containing cystosoral plasmodia. Stained with crystal violet. ×450.



FIGS. 58–62. Stages in vegetative nuclear division in zoosporangial plasmodia. Stained with crystal violet. Fig. 58. Interphase nucleus. ×1250. Fig. 59. Interphase nucleus. ×1200. Fig. 60. Cruciform stage. ×1200. Fig. 61. Final division stage. ×1250. Fig. 62. Final division stage. ×1200. Figs. 63–81. Stages in nuclear divisions in vegetative and sporogenic divisions of cystosoral plasmodia. Stained with crystal violet. ×1250. Figs. 63, 64. Interphase nuclei of vegetative divisions. Figs. 65–67. Cruciform stages of vegetative divisions in polar view. Figs. 68, 69. Cruciform stages of vegetative divisions in oblique view. Fig. 70. Dumbbell stage of cruciform division. Fig. 71. Final division of nucleolus in cruciform division. Fig. 72. Division of nucleus. Note four chromosomes present. Fig. 73. Ring of Saturn stage of cruciform division. Figs. 74–76. Zygotene (?), pachytene (?) and diplotene (?) respectively of the sporogenic division. See text. Figs. 77, 78. Late prophases of sporogenic divisions. Fig. 79. Anaphase of sporogenic division. Fig. 80. Anaphase of second sporogenic division in uninucleate thallus. Fig. 81. Telophase of second sporogenic division in uninucleate thallus. Fig. 82. Resting spores. ×1200. Fig. 83. Cystosorus. ×540. Fig. 84. Young zoosporangial plasmodia. One plasmodium with nuclei in cruciform stage, another with nuclei in dumbbell stage. Stained with crystal violet. ×1250. Fig. 85. Young infecting thallus in roots of host. ×880. Fig. 86. Young zoosporangial plasmodium. ×880. Fig. 87. Developing zoosporangial plasmodium. ×880. Fig. 88. Living multinucleate zoosporangial plasmodium with distinct nuclei. ×880. Fig. 89. Multinucleate zoosporangial plasmodium. ×605. Fig. 90. Young zoosporangia. ×605. Fig. 91. Zoosporangia with bulbous discharge pore. ×880. Fig. 92. Young zoosporangial plasmodia. ×605. Fig. 93. Biflagellate heterocont zoospore from zoosporangium. Stained using Löffler technique. ×1200.

the occurrence of a whip-lash on both flagella of a heterocont zoospore is unique in the fungi, it seemed that an investigation of the zoospores of the sporangia of *S. veronicae* would be interesting and informative. Zoospores were stained with Löffler's flagellar stain or gentian violet. These techniques have been used to demonstrate tinsels on the anterior flagellum and the whip-lash on the posterior flagellum of the zoospores of various aquatic fungi by Couch (1941). No tinsels were found on either flagellum, but a whip-lash was found at the tip of each flagellum, the anterior one being shorter than the posterior (Figs. 8, 9, 93).

Zoospores treated as above are spherical in shape and measure 5.6–7.0μ, with most 6.3μ, in diameter. The nucleus is in the center or slightly to one side of the spore body. The nucleolus is not conspicuous. Because the nucleus stains so densely, it was not possible, with the techniques used, to determine the point of attachment of the flagella, although these could be traced to the surface of the nucleus. There can be seen in some zoospores, in the finely granular cytoplasm,

darkly staining "lines" which appear to radiate from the nucleus and reach almost to the periphery of the cell. The short flagellum is 5.6–7.7μ long, and the long flagellum is 16.8–22.4 μ in length.

Morphology and Development of Cystosori.—Galls produced by *S. veronicae* in the shoot of *Veronica persica*, and to a lesser extent on *V. hederaefolia*, range in size from minute, almost microscopic swellings to tumors which may reach 3–4 cm. in length and 1 cm. in diameter. Usually they are 1–2 cm. in length and 0.25–0.5 cm. in diameter. In some cases infection is so severe that the entire shoot system appears to be one huge gall. If a cross section of gall material is made, it can be seen that each gall is generally composed of healthy cells, usually at the periphery of the gall, and masses of infected and modified cells with a few vessels showing here and there. The infected cells are extremely enlarged and may be multinucleate; they contain various stages in the development of the parasite (Fig. 57). Up to ten mature cystosori have been counted in one host cell. More detailed descriptions of the action of the parasite on the host cells of four other species of *Veronica* can be found in works by Maire and Tison (1909) and Blomfield and Schwartz (1910).

There is general agreement in the literature that the parasite invades the host plant in the apical meristem. Because of this the galls may involve almost the entire stem. In agreement with the observations of Blomfield and Schwartz (1910), no evidence was found to show that the parasite was able to move from cell to cell. Thus it would seem as they suggest, that the parasite is passively distributed by the repeated division of already infected cells, an opinion also expressed by Nawaschin (1899) in his paper on *Plasmodiophora*. In *V. persica*, infection seems to take place in the following manner. Some of the zoospores, liberated from the zoosporangia at times when soil water is plentiful, reach the surface of the soil. Because the host plants, after they have achieved some size, are recumbent, many apical meristems in the axils of leaves are available to the zoospores, which are able to swim for long periods of time under the cold conditions that are found during the winter months. These zoospores penetrate the host cells of the apical meristem and develop there into multinucleate plasmodia. Further development includes cleavage of plasmodia into incipient resting spores which are arranged in the form of hollow spheres or ellipsoids (Figs. 42, 52). The walls thicken and turn golden-brown upon achieving apparent maturity.

Circumstantial evidence in favor of the above conclusions may be had by observing infected plants, which in almost all cases will show that the lower axillary buds on the plants are infected. In addition, experimental evidence is available to substantiate the above argument, for galls were produced, under laboratory conditions, in the axillary buds of some *V. persica* plants by keeping the plants recumbent and by flooding the soil in the pots with water daily. Previously zoospores from sporangia had been added to the pots, and these were known to have infected the roots and to have produced zoosporangia there.

Maire and Tison (1909) claim that the plasmodium, by the time it has become eight-nucleate, may undergo schizogony and produce uninucleate or multinucleate meronts. Thus vegetative increase in the number of plasmodia takes place. No such happenings can be reported from this study. However, because of the intrinsic difficulty of such determinations on material of this nature, the above results are not considered to be definitive. On the other hand, Karling (1942) states "... that these workers [Maire and Tison] have never observed the actual splitting off of meronts, and their reports on the presence of schizogony are based only on the appearance of constricted plasmodia and the great abundance of uninucleate amoeba in infected cells."

Ivimey Cook (1933) states that the cystosori of *S. veronicae* are "enclosed in a universal membrane." Webb (1935) reports that a thin envelope covers the cystosorous after a thick wall is laid down around each spore. Other workers make no mention of such a membrane; no membrane, enveloping the cystosori of this isolate of *S. veronicae*, was found in the present study.

Living cystosori removed from freshly collected galls were found to be generally spherical and ellipsoidal and occasionally pyriform and irregular in shape. Spherical cystosori were 18.2–32.2μ in diameter, mostly 28.0μ in diameter; ellipsoidal ones 21.0 $\times$ 18.2μ–36.4 $\times$ 32.2μ in diameter. Cystosori were separated into individual spores by mounting them on a slide in concentrated sodium hydroxide and pressing on the cover slip. Individual resting spores were generally pyriform and pyramidal in shape, spherical on the surface forming the outside of the sphere and six-sided at the base which forms the inside of the hollow sphere (Figs. 42, 52, 82, 83). They measured 6.3 $\times$ 4.2 $\times$ 2.8–7.0 $\times$ 4.9 $\times$ 3.5μ.

Ledingham (1939) reports that he germinated

the resting spores of *S. veronicae*. According to Karling (1942), Professor J. T. Barrett germinated the resting spores of this parasite; he was unable to determine the number of flagella on the zoospores. After extensive periods of wetting, freezing and thawing, the cystosori of this isolate were germinated several times. It is thought that each resting spore produces one zoospore. The zoospores were found to be biflagellate and heterocont (Figs. 10, 11). The spore body was slightly smaller than the body of the zoospore from the zoosporangium, ranging from 4.0 to 4.8μ in diameter.

Cytological Studies

Ever since Nawaschin (1899) first observed that nuclear division during vegetative development of the plasmodium in *Plasmodiophora brassicae* differed from typical mitosis, cytological studies in this group for the past fifty years in the main have dealt with this type of nuclear division. Because of the minuteness of the nuclei, the ephemeral nature of these stages, the unselectivity of nuclear stains used, and the influence of protozoological findings, some conflicting information concerning these parasites can be found in the literature. However, students of this group do agree that during this type of nuclear division the nucleolus and nuclear membrane persist and the chromatin and nucleolus divide simultaneously, producing a cruciform figure during metaphase. It has been suggested by some workers that this cruciform division or "promitosis" or "protomitosis", as it often has been called, might be useful as a diagnostic character of the Plasmodiophorales.

Cytological Methods.—Gall and root material was killed and fixed either in Randolph's Modified Nawashin Fluid for twelve to twenty-four hours (Johansen, 1940), or in a modified Carnoy's Fluid for one hour (2 parts absolute alcohol:1 part acetic acid:1 part chloroform). After killing and fixation the material was transferred to 70 per cent alcohol for storage. Infected plant parts were embeded in paraffin after dehydration with tertiary butyl alcohol (Johansen, 1940), or they were dehydrated through 100 per cent ethanol and embedded using an alcohol-chloroform series: 3 parts absolute ethanol–1 part chloroform; 1 part absolute ethanol–1 part chloroform; 1 part absolute ethanol–3 parts chloroform, for 1–2 hours in each solution. The infected parts were then transferred to pure chloroform saturated with paraffin, and the chloroform was evaporated in a paraffin oven; the material was then transferred to fresh paraffin for two hours and then embedded. The paraffin blocks were sectioned at 8–10 microns, and the resulting ribbons were fixed to glass slides for future staining and study.

The paraffin was removed from the ribbons with xylene. The material was then hydrated by passing it through a decreasing ethanol series to pure water and stained in a 1 per cent aqueous solution of crystal violet for 15–30 minutes and then passed through the following series: water, 5 min.; iodine solution (iodine, 1 gm., potassium iodide, 2 gms.; water 300 cc.), 3 min.: water, rinse to indefinite time; 95 per cent ethanol, 5 sec.; absolute ethanol, 30 sec.; clove oil, 20 sec.; xylene, wash thoroughly; mount in balsam.

Semmens and Bhaduri (1939, 41) describe a cytological staining technique combining the Feulgen technique and light green which they found to be very satisfactory in differential staining of the nucleolus and chromosomes in some higher plant material. The schedule which they recommended has been slightly modified in that the Feulgen reagent used was prepared according to the method of Darlington and La Cour (1947), and the hydrolyzing HCl solution used was 1N instead of 12 per cent.

Cruciform Division in Zoosporangial Plasmodia.—The zoosporangial plasmodium begins as a naked uninucleate thallus in the roots of *Veronica persica*. By divisions of this infecting nucleus a multinucleate plasmodium is produced (Figs. 29, 31, 33). Because of the visible differences in the nuclear development of the plasmodia illustrated in figures 29, 31–33, and 84, it is concluded that each plasmodium remains an entity, at least during the early stages of development. The sequence of events to be reported here is found only in young plasmodia, and it seems that these stages are very rapidly passed through.

The interphase nucleus of the plasmodium has a very conspicuous nucleolus usually in the center of the nucleus. Chromatic strands and granules are seen within and toward the periphery of the nuclear membrane (Figs. 21, 34, 58, 59). As these nuclei begin to divide, and all nuclei in a single plasmodium divide simultaneously, there is an aggregation of the chromatin in the equatorial plane of the nucleus and around the persistent nucleolus which now begins to elongate in the direction of the poles. Thus the so-called cruci-

form stage is produced when the chromosomes form a discontinous ring around the elongating nucleolus and perpendicular to its long axis (Figs. 22–24, 60). Earlier workers describe this as a solid ring of chromatic material around the nucleolus. However, Horne (1930), Webb (1935), and Whiffen (1939) report the presence of individual chromosomes, and the observations recorded here are in agreement with their findings (Figs. 24, 60). The ring of chromosomes now divides, and the daughter chromosomes pass to the poles around the elongating nucleolus (Figs. 25, 26). This is the so-called dumbbell or double anchor stage. The nucleolus continues to elongate (Figs. 27, 28, 61, 62) finally breaking in the middle, each half taking part in the reorganization of the two daughter nuclei as the nuclear membrane completes its division.

As these vegetative nuclear divisions continue the plasmodium increases in size (Fig. 37), sometimes completely filling the host cell. Cruciform division, which occurs during the growth of the plasmodium, was not found to take place in the ensuing development of the zoosporangium. All divisions were of the typical mitotic type (Fig. 30). It was not possible to ascertain the number of these divisions because intermediate stages were not seen. Whiffen (1939) reports for the zoosporangia of *Octomyxa achlyae* that there may be three, four, or even five such divisions, and the spindles in all divisions are alike in size and form.

Cruciform Division in Cystosoral Plasmodia.—The interphase nuclei of the plasmodium are characterized by conspicuous chromatic granules, some of which seem to be connected with each other and are mainly concentrated immediately within the nuclear membrane; the nucleolus occupies usually a central position within the nucleus (Figs. 40, 41, 63, 64). Webb (1935) reports that these chromatic granules become aligned as the chromatic threads contract, and gradually four distinct chromosomes are seen. Similar stages have been seen in this study (Figs. 65–67); there appear to be four chromosomes present. Illustrations of cruciform divisions viewed obliquely are presented in figures 36, 68, 69, and from these it can be seen that the chromosomes do not form a continuous band or ring. This was reported by Milovidov in *Plasmodiophora brassicae* and by Webb in *Sorosphaera veronicae*.

The nucleolus continues to elongate and the chromosomes divide as shown in the photographs of early and late anaphase, often called the dumbbell stage of cruciform division (Figs. 50, 55). During late anaphase and telophase the nucleolus begins to constrict (Fig. 70), finally separating into daughter fragments. (Figs. 54, 71, 72). Lastly the nuclear membrane constricts and divides forming two daughter nuclei in which, at first, the chromosomes mass around the nucleolus. Figure 102 probably represents recent daughter nuclei which are beginning another division. The so-called "ring of Saturn stage" of cruciform division is seen; the nucleolus has not yet begun to elongate.

Further Development of the Cystosori.—The so-called transitional phase or akaryote stage of division was not recognized as such. These observations are in agreement with those of Milovidov who reported that in *Plasmodiophora* the chromatin never completely disappears from the nucleus. Webb observed in *Sorosphaera veronicae* that these changes were "of a simple nature, consisting of the degeneration of the large and conspicuous nucleolus . . ."; the chromatin reticulum remained clearly visible. The reports of other workers, especially Osborne (1911), that a fusion takes place during this stage, are based on a few apparently paired nuclei and on the fact that the nuclei appear to undergo reduction at sporogenesis, which presupposes a nuclear fusion. Horne (1930) for *Spongospora* and especially Webb (1935) for *Sorosphaera* found nuclear configurations which indicated to them that an alternation of four and eight chromosomes occurred. The haploid and diploid phases are supposedly separated by a fusion during the arkaryote stage, which is immediately followed by a reduction division. Whiffen (1939) concludes that *Octomyxa achlyae* has a similar life cycle. Although four chromosomes were counted in the vegetative stages of *S. veronicae*, no chromosome configurations were found which might indicate that a fusion and a reduction occur. However, certain configurations which are similar to those figured by Webb as reduction stages are included, and they may be interpreted as pointing up Webb's argument for an alternation of a haploid and a diploid number of chromosomes. The nucleolus becomes flattened against the nuclear membrane and the two groups of chromatin move together (Figs. 46, 74). These configurations are similar to those described by Webb as collapsed zygotenes, while figures 75 and 76 are similar to those described as pachytene and diplotene respectively.

According to Webb the nuclear membrane now disappears, and the chromosomes move to the equatorial plate; this heterotypic division is followed closely by the homeotypic division, interkinesis being of short duration. Metaphase (Figs. 45, 47), anaphase (Figs. 48, 79) and telophase (Figs. 49, 56) configurations found during the present study cannot be determined as belonging to either meiosis I or meiosis II.

Because no nuclear fusions have ever been found to occur in the Plasmodiophorales and reports of meiosis are strictly interpretive, it is suggested here that most data do not convincingly support the contentions that nuclear fusions and meiosis occur in the life cycle of these parasites. Thus another interpretation of the illustrations listed above in support of meiosis might be to present them as evidence for typical mitosis such as that which is thought to occur in zoospore formation. Then, after cruciform division has ceased, the so-called akaryote stage, which is really the prophase stage of the mitotic divisions which follow, occurs (Figs. 43, 44, 46, 77, 78). Metaphase (Figs. 45, 47), anaphase (Figs. 48, 79) and telophase (Figs. 49, 56) stages complete the division. Chromosome counts made during late anaphase from material which had been stained with the Feulgen technique show that the chromosome number is four, the same as that reported during cruciform division.

Two divisions occur. One is heterotypic and the other homeotypic, according to Webb. The present author observed nothing but typical mitotic divisions. During the first division the plasmodium cleaves into uninucleate portions (Figs. 42a, 53), in which the second division then takes place (Figs. 51, 80, 81). These incipient resting spores are associated in a hollow sphere (Figs. 42b, 52), and a thick wall is laid down around each resting spore (Fig. 82, 83).

Additional Observations on Cruciform Division —Because of the dual nature which has been attributed to the nucleolus of the Plasmodiophorales, i.e., it is said to be composed of two kinds of chromatin or to contain all or part of the chromatin of the nucleus, it seemed worthwhile to reinvestigate this matter. In addition to this is the question of whether or not true chromosomes are present in these organisms. Milovidov[2], using the Feulgen technique, demonstrated the presence of distinct chromosomes in the ring surrounding the nucleolus during vegetative division in *Plasmodiophora brassicae*. He further demonstrated that the nucleolus, unlike the chromosomes, gave no reaction for desoxyribonucleic acid. This indicates then that the nucleolus of this parasite is homologous with the nucleolus of other organisms.

Sorosphaera veronicae plasmodia were stained with Feulgen reagent and counterstained with light green according to a method devised by Semmens and Bhaduri (1939, 41). The results give positive indication that the persistent structure during cruciform division, which has been called the nucleolus, is truly this structure. Using this technique the nucleoli are stained a light to dark green and are easily distinguished from the purple chromosomes which respond to the Feulgen reaction. In interphase nuclei the nucleolus stains a bright green; during cruciform nuclear divisions, an elongated green nucleolus and a ring of purple chromosomes form the configurations so characteristic of these divisions.

Under the microscope it is very easy to distinguish between the colors of purple and bright green, but this difference is difficult to render with ordinary black and white photographic materials. However, the following illustrations are offered for what they do show. Figure 41 represents an interphase nucleus with a bright green nucleolus and purple chromatin, for the most part, around the internal periphery of the nuclear membrane. Figures 38 and 39 represent these differential stains much better. The contrast between the Feulgen-positive chromosome ring and the light green stained nucleolus is notable. These may be compared with like configurations shown in figures 35 and 36 which were stained with crystal violet. Figure 50, an anaphase of cruciform division, shows the darker rings of chromosomes surrounding a light green stained nucleolus.

It is very interesting to note in the work of Singh (1951), on nuclear division of nine species of free-living amoebae, that the karyosome "which should be called nucleolus" because it is Feulgen-negative, has been found to be void of true chromatin. Singh further states that the claim, which has been made that "peripheral chromatin" sometimes gives rise to chromosomes and sometimes disappears completely during division, [a phenomenon often claimed to be

[2] *See* J. S. Karling, 1942, The Plasmodiophorales, p. 5; and L. S. Olive, 1953, The structure and behavior of fungus nuclei, Bot. Rev. 19: 449.

characteristic of certain Plasmodiophorales] is now thought to be caused by, in part, unspecificity of the stains for chromosomal chromatin. Sole reliance on non-specific stains led to the fragmentation theory which offers the origin of chromosomal chromatin in the nucleolus.

SUMMARY

The discovery of zoosporangia in the life cycle of *Sorosphaera veronicae*, as determined by morphological, cytological and cultural studies, is described for the first time. The zoosporangia are located in the epidermal and sub-epidermal tissues of the roots, root hairs excepted; the cystosori are found only in the shoot system. Zoospores, from resting spores found in the soil, penetrate root cells, developing there into zoosporangial plasmodia. Mature zoosporangia discharge biflagellate heterocont zoospores—with a whip-lash on each flagellum, which may reinfect root cells. It is assumed that these zoospores, at times when soil water is plentiful, reach the surface of the soil. Because of the recumbent nature of the host plants, many apical meristems are available to the zoospores, which may enter them and form cystosoral plasmodia. Mature cystosori are liberated in the soil upon the death and decay of the infected host plants. Cystosori are not produced in the roots.

Cytological investigations indicate that, during the early stages of plasmodial development in *S. veronicae*, a vegetative nuclear division as characterized below is found. Chromosomes are present; they are not derived wholly or in part from the nucleolus. Division of the chromosomes takes place within the nuclear membrane. The nucleolus is persistent within the ring of chromosomes. Gradually it divides into two portions as the chromosomes reach telophase; the nuclear membrane then divides.

Veronica persica and *V. perigrina* are reported as new hosts for *Sorosphaera veronicae*.

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EFFECTS OF GIBBERELLIC ACID ON THE GROWTH AND DEVELOPMENT OF *XANTHIUM PENSYLVANICUM*[1]

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The gibberellins are a group of compounds which cause a number of striking responses in plants, the most universal one being the marked promotion of stem growth. Other effects which have been reported include the alteration of the size and shape of leaves (Gray, 1957), reversal of genetic dwarfism (Brian and Hemming, 1955), replacement of low temperature (Lang, 1956) and long day photoperiod (Wittwer and Bukovac, 1957) requirements for flowering, replacement of red light requirement for lettuce seed germination (Kahn *et al.*, 1957), and the induction of parthenocarpic fruit development in tomato (Wittwer *et al.*, 1957). Other reports on the effects of gibberellin on plants have recently been reviewed by Stowe and Yamaki (1957).

This paper reports the results of experiments with gibberellic acid on *Xanthium pensylvanicum* Wallr. The investigation was undertaken to determine if gibberellic acid could replace short day photoperiodic requirements for this species, and to determine the effects of gibberellic acid and photoperiod on the over-all growth of the plants as expressed by shoot height and fresh and dry weight determinations of all aerial plant parts.

Materials and Methods.—Seeds of *Xanthium pensylvanicum* were removed from the burs and sown singly in two-inch pots of soil in a greenhouse on April 11, 1957, the resulting seedlings being transplanted into individual four-inch pots two weeks later. The plants were maintained on long (16 hr.) days from the time the seed was sown until the time of treatment (May 9, 1957).

When the plants were four weeks old, twenty-four were selected for the experiment and these were divided into two groups of twelve plants each. A set of six plants from each group was treated with gibberellic acid and the other six plants in each group served as controls. After treatment, one group of plants was placed under short (8 hr.) days and the other group was maintained under long days. One ml. of 10^{-3} M gibberellic acid in aqueous 0.25 per cent lauryl sulfate solution was applied in small drops to the upper surfaces of the expanded leaves of each of the treated plants. The control plants were treated similarly with 0.25 per cent aqueous solution of lauryl sulfate only, which served as a wetting agent.

Measurements of the height of the stems above the cotyledonary node and counts of the total number of nodes of each plant, were made at the time of treatment on May 9 two weeks after treatment on May 23, and at the time the experiment was terminated on June 19. The May 23 measurements are not considered in this paper. The following additional data were taken when the experiment was terminated: the total number, fresh weight and dry weight of the leaves of each set of plants; the fresh weight and dry weight of the stems of each set of plants; and the number, fresh weight and dry weight of the burs of each set of plants which had been maintained on short days.

Results and Discussion.—The average stem lengths for each set of plants were calculated, growth of the stems of each set of plants during the experiment being determined by subtracting the average stem length at the time of treatment from the average stem length at the end of the experiment. These data are presented in Table 1.

Plants treated with gibberellic acid and then maintained on long days showed a 158 per cent increase in stem length over that of the controls. Similarly treated plants maintained on short days showed a 243 per cent increase in stem length over that of the controls. This effect of gibberellic acid on stem elongation of *Xanthium* is certainly not surprising, since nearly every paper dealing with the effects of gibberellic acid on other species has reported a similar growth response. Marth *et al.* (1956) have reported increases in stem length in some species of as much as 300 per cent over that of controls.

Gibberellic acid also increased the number of visible nodes per plant, a result which has not been found in some other species which have been

[1] The gibberellic acid used in this experiment was supplied through the courtesy of Dr. Edwin F. Alder of the Lilly Research Laboratories.

TABLE 1

Mean Stem Growth of Xanthium *Plants During Experiment, cm.*

	Long Day	Short Day	LD-SD
Controls	47.0	20.9	26.1
Gibberellic Acid	74.2	50.8	23.4
GA-C	27.2	29.9	—

investigated. The mean number of nodes under short days was 8 for treated plants as compared with 7 for the controls, while under long days the treated plant averaged 17 nodes as compared with only 10 for the controls. This evidence suggests that gibberellic acid increased the rate of cell division in the growing stems. Kato (1955) concluded, from anatomical studies of stems of bean seedlings treated with gibberellin, that the growth resulting from the treatment was a consequence of accelerated cell elongation rather than of cell multiplication. Many other investigators have also stated that gibberellin-induced stem elongation is primarily the result of increased cell elongation (e.g. Stowe and Yamaki, 1957). However, Sachs and Lang (1957) have recently proven that gibberellin definitely stimulates cell division in the subapical region of nonvernalized biennial *Hyocyamus niger* rosettes. Also, Bradley and Crane (1957) have demonstrated gibberellin-stimulated division of cambial cells in stems of apricot spur shoots.

Plants maintained on short days formed fewer nodes than those maintained on long days. This was the result of the photoinduction of flowering and the consequent termination of vegetative growth in all of the short day plants. Flower buds were first visible eleven days after the plants were placed on short days. All of the plants maintained on long days continued to grow vegetatively throughout the experiment. Thus, gibberellic acid treatment did not replace the short day requirement for the flowering of this species. Wittwer and Bukovac (1957) and others have reported gibberellin-induced flowering of several long day annuals kept under non-inductive photoperiods, but there have been no reports of gibberellin-induced flowering of short day plants maintained on long days. However, in a subsequent experiment reported on elsewhere (Greulach and Haesloop, 1958), the writers have shown that gibberellic acid may greatly accelerate the development of flower primordia in *Xanthium* once they are initiated, if the plants are given fewer photoinductive cycles than employed in this experiment.

The leaves produced by the long day-gibberellic acid treated plants were generally smaller and narrower than those produced by control plants. Gibberellic acid has been shown to cause variation in leaf form in several other species of plants (e.g. Gray, 1957). The number of leaves per plant was greater on the gibberellic acid treated plants as a result of the development of more nodes by these plants.

TABLE 2

Effect of Gibberellic Acid and Photoperiod on the Mean Weight per Plant, grams, of Various Organs of Xanthium

	Long Day		Short Day	
	Controls	Gib. Acid	Controls	Gib. Acid
Fresh Weight				
Stems	20.97	25.92	4.83	6.90
Leaves	16.67	16.39	17.55	10.56
Burs	—	—	9.60	8.52
Total	37.64	42.31	31.98	25.98
Dry Weight				
Stems	4.62	5.07	0.72	1.19
Leaves	2.66	2.61	2.12	1.45
Burs	—	—	2.54	1.72
Total	7.28	7.68	5.38	4.36

TABLE 3

Effect of Gibberellic Acid and Photoperiod on the Mean Weight per Unit Structure, grams, of Various Organs of Xanthium

	Long Day		Short Day	
	Control	Gib. Acid	Control	Gib. Acid
Fresh Weight per:				
Internode	2.62	1.62	0.81	0.99
Leaf	2.38	1.23	2.19	1.29
Bur	—	—	0.19	0.16
Total	5.00	2.85	3.19	2.45
Dry Weight per:				
Internode	0.58	0.32	0.12	0.17
Leaf	0.38	0.20	0.20	0.18
Bur	—	—	0.05	0.03
Total	0.96	0.52	0.37	0.38

The fresh and dry weights of the stems, leaves, and burs of all plants were determined at the end of the experiment. In Table 2 these data are presented on a per plant basis and in Table 3 they are presented on a unit structure basis. The fresh and dry weights of plants maintained on long days after treatment were higher than those maintained on short days, probably because the plants on long days not only remained vegetative but received much longer daily periods for photosynthesis than those on short days. Both the fresh and dry weights of the leaves of gibberellin-treated plants were less than those of the controls. The leaf weights, on a per plant basis, of gibberellin-treated plants on long days were only slightly less than those of the controls, but the gibberellin plants developed nearly twice as many leaves as did the controls on long days. The fresh and dry weights of the stems of treated plants were greater than those of the controls. However, on long days, the weights per internode of treated plants were less than control internodes because the treated plants developed more internodes.

The burs produced by the gibberellin-treated plants were smaller and more immature than those on the control plants at the end of the experiment. However, none of the burs from either the treated or control plants were mature when the experiment was ended. The smaller size of the burs on the treated plants is reflected by their lower fresh and dry weights in Tables 2 and 3. The treated plants on short days produced a total of 329 burs or an average of 55 burs per plant. The controls on short days produced a total of 304 burs or an average of 50 burs per plant.

SUMMARY

Gibberellic acid caused a marked increase in stem elongation of *X. pensylvanicum* plants maintained on both long and short days and also an increase in the number of nodes per plant. Leaves of treated plants were smaller, more numerous, and of less fresh and dry weight than those of the controls. The fresh and dry weights of the stems were increased by gibberellin. The weights of burs, however, were decreased by treatment. Gibberellic acid did not replace the short day requirement for flowering of *X. pensylvanicum* under the conditions of this experiment.

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SUGGESTIONS TO CONTRIBUTORS

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DR. JOHN N. COUCH, *Editor*
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(5) BRAUER, A., AND N. MACON. Amer. Jour. Math. **71**: 349–361. 1949.
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